The Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome (TB-IRIS): recent advances in clinical and pathogenesis research

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Abstract

**Purpose of the review:**
Anti-retroviral therapy (ART) is an essential, life-saving intervention for HIV infection. However, ART initiation is frequently complicated by the tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) in TB endemic settings. Here, we summarise current understanding highlighting the recent evidence.

**Recent findings:**
The incidence of paradoxical TB-IRIS is estimated at 18% (95% CI 16-21%), higher than previously reported and may be over 50% in high-risk groups. Early ART initiation in TB patients increases TB-IRIS risk by greater than two-fold, but is critical in TB patients with CD4 counts <50 cells/µl because it improves survival. There remains no validated diagnostic test for TB-IRIS, and biomarkers recently proposed are not routinely used. Prednisone initiated alongside ART in selected patients with CD4 ≤100 cells/µl reduced the risk of paradoxical TB-IRIS by 30% in a recent randomised-controlled trial and were not associated with significant adverse effects. Effective also for treating paradoxical TB-IRIS, corticosteroids remain the only therapeutic intervention for TB-IRIS supported by randomised-controlled trial data. TB-IRIS pathogenesis studies implicate high antigen burden, innate immune cell cytotoxicity, inflammasome activation and dysregulated matrix metalloproteinases in the development of the condition.

**Summary:**
Specific biomarkers would aid in identifying high-risk patients for interventions and a diagnostic test is needed. Clinicians should consider prednisone for TB-IRIS prevention in selected patients. Future research should focus on improving diagnosis and investigating novel therapeutic interventions, especially for patients in whom corticosteroid therapy is contraindicated.

**Keywords:** HIV-1 infection; immune reconstitution inflammatory syndrome; paradoxical; tuberculosis; unmasking.
### Introduction

HIV-associated TB is common, with an estimated 1.4 million cases and 374,000 deaths annually [1]. In parts of sub-Saharan Africa, around 60% of TB patients are HIV-co-infected [1]. Anti-retroviral therapy (ART) is an essential, life-saving intervention for HIV, but HIV-infected patients starting ART are at high risk of tuberculosis-associated immune reconstitution inflammatory syndrome (TB-IRIS) in TB endemic settings. TB-IRIS is an acute inflammatory condition that presents with worsening, or development of new, tuberculosis pathology in a patient already on TB treatment after starting ART (paradoxical TB-IRIS), or a new diagnosis of TB with a particularly acute, inflammatory presentation after starting ART (unmasking TB-IRIS). Rapid restoration of immunity after ART, with exaggerated inflammatory responses to *Mycobacterium tuberculosis* (Mtb) antigens, underlies this condition although the pathophysiology is incompletely understood.

In a systematic review, Namale *et al* collated studies published before May 2014 reporting incidence, clinical features, management and outcomes of paradoxical TB-IRIS [2], including 40 studies, 7,789 patients at risk and 1,048 TB-IRIS cases. Studies were from Africa, Asia, Europe, North and South America. Here, we discuss key findings in subsequently published literature, on TB-IRIS epidemiology, outcomes, management, prevention, diagnosis and pathogenesis. We focus on paradoxical TB-IRIS, the most common form of HIV-associated IRIS and most frequently studied. Unmasking TB-IRIS is discussed in a separate section. Knowledge to date, with reference to key review articles and recent original research papers is summarised in Table 1.
**Table 1: Paradoxical TB-IRIS – knowledge summary**

<table>
<thead>
<tr>
<th>Knowledge summary</th>
<th>Key references</th>
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<tbody>
<tr>
<td><strong>Incidence</strong></td>
<td>Reviewed in [2] Recent cohort described in [3]</td>
</tr>
<tr>
<td>Adults overall: 18% (95% CI 16-21%), with a range of 4-54%; higher rates in patients with lower CD4 counts (up to 57% in patients with CD4 count &lt;200 cells/µL).</td>
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<td>South African children: 6.7% reported in a recent prospective study.</td>
<td>[4]</td>
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<tr>
<td><strong>Risk factors</strong></td>
<td>Reviewed in [2]</td>
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<tr>
<td>Low CD4 count at ART initiation;</td>
<td>Meta-analyses reported in [5], [6]</td>
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<tr>
<td>High HIV viral load at ART initiation.</td>
<td></td>
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<td>Shorter time between TB treatment initiation and ART initiation.</td>
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<tr>
<td>Disseminated TB/high mycobacterial load.</td>
<td>[3], [7]</td>
</tr>
<tr>
<td><strong>Clinical presentation</strong></td>
<td>Reviewed in [2]</td>
</tr>
<tr>
<td>Systemic, pulmonary and lymph node presentations most common.</td>
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<tr>
<td>In a recent study, median days to symptom onset reported as 6 (range 1-23).</td>
<td>[3]</td>
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<tr>
<td><strong>Mortality</strong></td>
<td>Reviewed in [2]</td>
</tr>
<tr>
<td>All-cause mortality rate of 7% (95% CI 4-11%) and IRIS-attributable deaths of 2% (95% CI 1-3%).</td>
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<tr>
<td>Higher mortality in CNS TB-IRIS</td>
<td>Reviewed in [8]</td>
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<tr>
<td><strong>Pathogenesis</strong></td>
<td>Reviewed in [9]; see also [3], [10], [7]</td>
</tr>
<tr>
<td>Innate immune cell activation, including neutrophils, monocytes and NK cells;</td>
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<td>Antigen-specific upregulation of cytotoxic mediators</td>
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<tr>
<td>Inflammasome activation;</td>
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<tr>
<td>Hypercytokinaemia (including IL-1β, IL-6, TNF-α) and MMP upregulation/secretion.</td>
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<td><strong>Treatment</strong></td>
<td>Randomised-controlled trial reported in [11]</td>
</tr>
<tr>
<td>Prednisone (1.5mg/kg for 2 weeks followed by 0.75mg/kg for 2 weeks) for treatment of paradoxical TB-IRIS reduced length of hospital admission and number of therapeutic procedures required, and improved symptoms in paradoxical TB-IRIS.</td>
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<tr>
<td>Consensus is not to stop ART, but to investigate fully for alternative causes, and provide symptomatic treatment.</td>
<td>Reviewed in [9]</td>
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<td><strong>Prevention</strong></td>
<td>[12]</td>
</tr>
<tr>
<td>Prednisone (40 mg daily for 2 weeks, followed by 20 mg daily for 2 weeks) from ART initiation reduces the risk of future paradoxical TB-IRIS by 30%.</td>
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<tr>
<td>Do not delay ART initiation beyond 2 weeks after TB treatment initiation in patients with CD4 count &lt;50 cells/mm³, unless CNS TB diagnosed (then delay 4-8 weeks). Early ART improves survival in patients with CD4 &lt; 50 cells/mm³ even though it increases TB-IRIS risk &gt; 2-fold.</td>
<td>Meta-analyses reported in [6], [5]</td>
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Definitions

As there are no validated diagnostic tests for TB-IRIS, diagnosis is clinical. The International Network for the Study of HIV-associated IRIS (INSHI) consensus definitions of paradoxical and unmasking TB-IRIS are the most commonly used and have been validated [13, 14]. The Aids Clinical Trials Group definition (see https://actgnetwork.org/IRIS_Case_Definitions) and IMPAACT trial definition (see https://www.impaactnetwork.org for Adolescent and Paediatric cohorts) have been used in research settings. The schematic in Figure 1 summarises the different terms used and the relationship to TB treatment and ART initiation. The case definitions provide sub-classification into confirmed and probable paradoxical TB-IRIS according to the extent in which other possible causes of symptoms have been adequately excluded. ART-associated TB is a broad term that encompasses new TB diagnoses in patients who have recently commenced ART, including unmasking TB-IRIS, with the recognition that new TB diagnoses are frequently made in patients who have recently commenced ART but not all have the features of unmasking IRIS [13]. Two illustrative cases of paradoxical TB-IRIS cases are presented in Figure 2.
Adapted from INSHI definition. TB-IRIS can also occur when ART is re-initiated after stopping ART and when changing from a failing regimen to a new effective ART regimen. ART=anti-retroviral therapy.

*Major criteria for Paradoxical TB-IRIS: i) new/enlarging LN, cold abscess, or other focal tissue involvement; ii) new/worsening radiological features of TB; iii) new or worsening central nervous system tuberculosis; iv) new or worsening serositis. Minor criteria for Paradoxical TB-IRIS: i) new/worsening constitutional symptoms; ii) new/worsening respiratory symptoms; iii) new/worsening abdominal pain plus peritonitis/hepatomegaly/splenomegaly/abdominal adenopathy.
Patient A was a 29-year-old male, with a CD4 count of 14 cells/μl, who had been on TB treatment for one month. He complained of loss of appetite, 4 kg weight loss, and recurrence of cough and chest pain two weeks after starting ART. Chest radiograph shows extension of the left hilar infiltrate. His c-reactive protein had increased from 11 mg/l before the start of ART to 292 mg/l. His symptoms spontaneously resolved two weeks later.

Patient B was a 36-year-old male, with a CD4 count of 73 cells/μl, who had been on TB treatment for 3 weeks. One week after starting ART he complained about poor appetite, gradually worsening with dyspnoea, cough, night sweats, diarrhoea, vomiting and fatigue. He was tachypnoeic, with a temperature of 38°C. Chest radiograph showed an increase in hilar and paratracheal lymphadenopathy. His c-reactive protein had increased from 4 mg/l before the start of ART to 120 mg/l. He was started on prednisone treatment, resulting in complete resolution of his symptoms over the next few weeks.
**Paradoxical TB-IRIS**

**Incidence and risk factors**

Recently reported cohort studies of adult patients in Africa and India have demonstrated high TB-IRIS incidence rates (19-57%) with the highest rates in patients with low CD4 counts [3, 15-17]. A study of HIV-infected and -uninfected patients with TB meningitis in India, reported paradoxical reactions in 11/13 (84.6%) HIV-infected patients, but did not report on the temporal relationship to ART initiation [18]. A recent cohort study of 104 South African children was the first to prospectively study paradoxical TB-IRIS in children concluding that the incidence of paradoxical TB-IRIS in children was low. Paradoxical TB-IRIS occurred in 7/104 (6.7%) children studied [4]. A recent trial of urgent (<48h) versus post-stabilisation (7-14 days) ART initiation in hospitalised children in Kenya, reported IRIS incidence as a primary outcome measure, using IMPAACT definitions of IRIS. 27/179 children had a diagnosis of suspected TB at enrolment and nine children were on TB treatment at enrolment. 18 patients had suspected TB-IRIS during 6 months follow up, although it is not stated whether these were unmasking or paradoxical TB-IRIS [9].

Baseline low CD4 count and high HIV viral load have been associated with an increased risk of TB-IRIS in prospective studies, including randomised controlled trials evaluating the optimal time for ART initiation in HIV [2]. The most recently published was a study of 478 patients in Ethiopia, starting ART at week 1, 4 or 8 weeks of TB treatment, powered for mortality outcome at 48 weeks. No survival benefit was evident with ART initiation at week 1 and TB-IRIS was more frequent in patients with early vs later ART initiation, with an incidence of 15 (95% CI 9-24), 5 (95% CI 2.3-11.7) and 0 per 100 person years in those who commenced ART at week 1, 4 and 8 respectively (p=0.001) [19].
Two meta-analyses of randomised-controlled trials (RCTs) on the optimal time to initiate ART following commencement of TB treatment in HIV-associated TB have reported combined relative risks of TB-IRIS of 2.19 (95% CI 1.77-2.70) and 2.31 (95% CI 1.87-2.86) for early (up to four weeks) vs late (8-12 weeks) ART initiation [5, 6]. In patients with a CD4 count below 50 cells/mm³ a mortality benefit was apparent with early ART, favouring ART initiation within 2 weeks following TB treatment in such patients. However, a relatively high incidence of TB-IRIS can be expected in these patients who commence ART at 2 weeks of anti-tuberculous therapy – the risk was elevated over two-fold with early ART.

Our cohort study followed 47 TB patients (with baseline CD4<200 cells/µL) commencing ART, investigating immunopathology caused by matrix metalloproteinases [3]. The incidence of paradoxical TB-IRIS was 57%. Patients who went on to develop TB-IRIS had higher plasma MMP-8 and procollagen III N-terminal propeptide (PIIINP, a matrix degradation product released during collagen turnover) and evidence of an increased systemic inflammatory response (higher heart rates, higher respiratory rates) but lower lymphocyte counts, at TB diagnosis, compared to those that did not develop TB-IRIS. Urine lipoarabinomannan (LAM, a marker of renal TB indicating disseminated infection in patients with advanced HIV) was more often positive in TB-IRIS patients (adjusted OR = 10.9). Marais et al reported increased proportions of CSF culture positivity and persistently positive CSF cultures in patients who developed TBM-IRIS relative to those that did not [7]. However, a recent study of 90 patients with baseline CD4 count <250 cells/mm³, starting ART, nested within an RCT of early vs late ART, did not find an association between positive blood cultures for TB and TB IRIS diagnosis [20].
The question of whether integrase inhibitors increase the risk of paradoxical TB-IRIS as they result in a more rapid decline in HIV viral has arisen with increasing use of integrase inhibitors in first line ART regimens. Observational studies have reported an association with all-cause IRIS [21]. A recent meta-analysis addressing Dolutegravir use did not find an association with IRIS [22, 23]. Clinical trials have not demonstrated an increased risk of TB-IRIS [24]. This question is being further addressed in ongoing trials.

In summary, paradoxical TB-IRIS frequently complicates ART initiation in HIV-associated TB, with a high incidence in selected patients. More advanced immunosuppression, more inflammatory TB presentation, disseminated TB and persistent culture positivity, and a shorter time to ART initiation from TB treatment have all been associated with increased TB-IRIS risk and together suggest that increased mycobacterial burden permitted by advanced immunosuppression are causally associated with development of TB-IRIS.

**Outcomes**

Overall mortality from paradoxical TB-IRIS appears to be relatively low but may be confounded by under-diagnosis and lack of reporting [2]. Recent cohort studies have reported few or no deaths due to TB-IRIS. The most recently published RCT of timing of ART initiation in TB patients reported only 1 TB-IRIS case amongst 64 deaths [19]. However, in a pooled analysis of RCTs that reported TB-IRIS events, the estimated relative risk of death from paradoxical TB-IRIS in early ART arms vs late arms was 6.94 (95% CI 1.26-38.22) with an event rate of 0.78% (9/1153 cases) in the early arms versus 0/1119 in the late arms [5]. Central nervous system TB-IRIS carries a particularly high mortality risk, up to 30% [7]. In our cohort
study of 47 patients, hospital admission was required in 13/49 (45%) TB-IRIS patients compared to 1/18 (6%) non-IRIS controls (p=0.007). There were no deaths from TB-IRIS in this study, performed in an experienced research centre with close clinical monitoring [3].

**Diagnostic and prognostic biomarkers**

In a prospective observational study of 170 TB patients with CD4 counts <125 cell/µl, starting ART in Botswana, 33 (19%) patients developed paradoxical TB-IRIS and 18 (11%) patients died, but there was only 1 (3%) death amongst TB-IRIS patients, reported as occurring after the resolution of TB-IRIS symptoms [17]. This study evaluated 26 biomarkers in plasma by Luminex, in baseline pre-ART samples with respect to an eventual diagnosis of paradoxical TB-IRIS or death. Lower growth factor and Th1 cytokine responses, and lower concentrations of the pro-inflammatory cytokine IL-17 were associated with TB-IRIS whereas higher baseline pro-inflammatory cytokines, tumour necrosis factor-α (TNF-α) and IL-6, in addition to MCP-1 and EOTAXIN, were associated with death, suggesting different pathophysiological mechanisms leading to TB-IRIS and early mortality due to TB, following ART initiation.

A study of 267 patients nested within the CADIRIS trial (see below), evaluated 20 biomarkers at ART baseline to investigate an association with all-cause IRIS [25]. There were 62 IRIS cases diagnosed using ACTG definitions, of which 19 (34%) were TB-IRIS (14 paradoxical, 5 unmasking). TB-IRIS was associated with a distinct biosignature comprising elevated c-reactive protein, soluble CD14 and interferon-γ (IFN-γ) and lower haemoglobin. These factors were combined into a score that allowed prediction of TB-IRIS vs no-IRIS with a sensitivity of 71.4% (0.52-88.7) and a specificity of 73.2% (57.1-85.9).
A Malaysian study comparing a combined group of patients with paradoxical and unmasking TB-IRIS (n=15) to controls with TB but no IRIS (n=14) and those without TB or IRIS (n=15), using INSHI definitions, found elevated IL-18 and CXCL-10 levels prior to ART to be predictive of TB-IRIS [26]. In a validation cohort in India, baseline IL-18 predicted paradoxical TB-IRIS with an AUC = 0.742, p=0.004 (no confidence intervals reported) [26]. One study investigated the predictive value of antigen-specific cytokine responses for TB-IRIS prediction and found it to be of little value [27].

**Management**

Management of paradoxical TB-IRIS includes investigations to rule out other diagnoses (e.g. sepsis), supportive management (e.g. IV fluids for hypotension), symptomatic treatment (e.g. analgesia, anti-emetics), surgical or percutaneous interventions (e.g. abscess drainage) and inhibition of excessive immune responses (corticosteroids). It is critical to exclude drug-resistant TB, which is an important cause of clinical deterioration in HIV-associated TB, and which can manifest with TB-IRIS features that are clinically indistinguishable from IRIS seen in drug-sensitive TB [28]. ART interruption is not recommended.

Corticosteroids remain the only treatment for paradoxical TB-IRIS whose use is supported by randomised-controlled trial data [11]. Other immunomodulatory therapies have been the explored in case reports and case series including several reports of anti-TNF-α therapy, and one report of intravitreal anti-VEGF (Bevacivumab) therapy in ocular TB-IRIS [29-32].

**Prevention**
Two double-blind randomized, placebo-controlled studies have assessed strategies to prevent TB-IRIS in adults. The CADIRIS study assessed the efficacy of the CCR5 blocker maraviroc in reducing all-cause IRIS, including TB-IRIS, in patients with CD4 count ≤100 cells/µl. Time to an IRIS event by 24 weeks was the primary outcome [33]. No difference in proportion of TB-IRIS was found between the maraviroc and the placebo arm.

The PredART trial assessed the efficacy and safety of prophylactic prednisone in preventing TB-IRIS in patients who are identified as being at high risk for paradoxical TB-IRIS [12]. Inclusion criteria included CD4 count ≤100 cells/µl, microbiologically confirmed TB or clinical diagnosis with symptomatic response to anti-tuberculosis treatment, and starting ART within 30 days after starting anti-tuberculosis treatment [12]. Exclusion criteria included Kaposi’s sarcoma, neurological or pericardial tuberculosis, rifampicin-resistant tuberculosis, and hepatitis B surface antigen positivity. The primary endpoint was the development of paradoxical TB-IRIS (according to the INSHI consensus definition) within 12 weeks after starting ART adjudicated by an independent committee. 240 participants were randomized 1:1 to receive either prednisone (40 mg daily for 2 weeks, followed by 20 mg daily for 2 weeks) or identical placebo within 48 hours of starting ART. Prophylactic prednisone reduced the risk of paradoxical TB-IRIS by 30% (56/120 in the placebo arm versus 39/120 in the prednisone arm, p=0.02), corresponding with an absolute reduction in incidence of 14.2%.

Prednisone was safe, with no statistically significant difference in grade 4 adverse events, severe infections, malignancies, death, corticosteroids side-effects, or immunological and virological outcomes at week 12, between the prednisone and the placebo arm. There did not appear to be “breakthrough cases” when the 28-day course of prednisone was stopped.
Prednisone also reduced the incidence of more severe TB-IRIS, judged from the number of patients with TB-IRIS fulfilling at least one INSHI major criterion or prescribed prednisone treatment of TB-IRIS; proportions for both these secondary outcomes were also reduced in the prednisone arm.

Further study is required to conclude whether prednisone would benefit patients not included by the enrolment criteria. However, based on this result, we recommend preventive treatment with prednisone, at the doses above, for HIV-infected TB patients with a CD4 nadir $\leq 100$ cells/µl, who have had hepatitis B and Kaposi’s sarcoma excluded, and who are not diagnosed with rifampicin resistant TB and who are symptomatically improving on TB treatment prior to ART. A similar recommendation has been included in the 2017 European AIDS Clinical Society guidelines [34].

**Pathogenesis**

Recent evidence has implicated exaggerated cytotoxic responses, excessive pro-inflammatory innate immune responses mediated by inflammasome activation, and MMP-driven tissue damage in TB-IRIS pathogenesis. These processes are likely to be inter-related. A detailed review of the pathogenesis of TB-IRIS is beyond the scope of this review. However, here we detail key recent studies that have enhanced understanding.

We investigated transcription profiles of Mtb-stimulated peripheral blood mononuclear cells (PBMC) of TB-IRIS and controls who did not develop TB-IRIS, finding cytotoxic mediators perforin and granzyme B to be amongst the top differentially regulated genes. Correspondingly granzyme B was increased in serum of TB-IRIS patients but reduced in
prednisone treated patients. We found elevated CD3+Vα24+ cell populations in TB-IRIS patients suggesting Natural Killer T (NKT) cells may play a role in TB-IRIS. A previous report found increased Natural Killer (NK) cell degranulation predicted TB-IRIS and together these studies support a role for aberrant cytotoxic responses [35]. Further studies exploring the clinical implications of these findings are required.

Lai et al performed transcriptomic analysis of whole blood from 17 TB-IRIS patients and 15 non-IRIS controls in a longitudinal study, pre-ART, at 0.5 weeks on ART and at 2 weeks on ART initiation (time of IRIS onset) [36]. The early pre-IRIS transcriptomic signature on ART in patients who developed TB-IRIS was enriched for genes associated with innate immunity, including the JAK family of kinases involved in IL-6 signalling pathways, interferon signalling, pattern recognition receptors and macrophage function. At the time of TB-IRIS onset, TLR receptor, TREM-1 signalling, and the role of pattern recognition receptors were amongst the most upregulated pathways indicating innate immune function to be at the centre of divergent immune responses. This was validated by plasma measurement of cytokines at the time of TB-IRIS onset: IL-12p40, IL-6, TNF-α and IFN-γ were found to be significantly increased compared to non-IRIS controls. Increased IL-1β, IL-1α, caspase-1 and caspase-5 secretion from heat-killed H37Rv Mtb stimulated PBMC from TB-IRIS patients compared to non-IRIS controls, suggested increased inflammasome activation in TB-IRIS.

Excessive MMP activity is implicated in inflammatory pathology in TB-IRIS and may be corticosteroid modulated [37]. In our recent study, plasma MMP-8 (neutrophil collagenase) was most significantly elevated in TB-IRIS patients, at TB diagnosis and at the time of TB-IRIS onset, compared to controls [3]. Plasma MMP-8 correlated with peripheral blood neutrophil
count, suggesting it may be neutrophil-derived. PIIINP was elevated in TB-IRIS patients at TB diagnosis and at the time of TB-IRIS. The previously described Botswanan cohort study found increases in plasma MMP-8 on ART to be associated with TB-IRIS, and abnormal pulmonary function following TB treatment, although intervention studies are required to prove a causal link [16].

Neutrophils are elevated, activated and found at the site of caseous necrosis in human TB-IRIS [10]. In transcriptional analysis of unstimulated PBMC from TB-IRIS patients, the most upregulated transcripts in TB-IRIS patients vs controls implicated increased neutrophil activity (S100A9, NLRP12, COX-1 and IL-10), and this was supported by elevated neutrophil elastase and human neutrophil peptides (HNP) 1-3 in plasma of TB-IRIS patients. These data suggest that neutrophil influx to the site of disease, activation and early cell death occur in TB-IRIS patients leading to local necrosis and tissue destruction.

In the context of advanced HIV-1 and Mtb infection, impaired Mtb antigen presentation by antigen presenting cells may permit excessive mycobacterial replication pre-ART. This may be contributed to by direct effects of the HIV-1 virus and poor T cell help. Differential recovery of T cell subsets, particularly with respect to activation and memory cell responses have been associated with TB-IRIS [38, 39]. However, the studies described above highlight the critical role for early innate immune responses in the excessive inflammation underlying TB-IRIS. Inflammasome activation and MMP activity are potential targets for future host-directed therapies.

**Unmasking TB-IRIS**
Although ART has a major impact on reducing the risk of TB in HIV infected individuals, TB remains the most frequently diagnosed opportunistic infection after ART initiation, particularly in high TB burden settings. The risk of developing TB is highest within the first three months after starting ART [40]. There may be delayed immune recovery after initiation of ART, increasing the period of high risk for HIV-associated TB. Additionally there may be subclinical TB (sputum culture positivity in asymptomatic individuals), prevalent in resource-limited settings such as sub-Saharan Africa where current screening tools perform sub-optimally for active case finding [41, 42]. Thus, a substantial number of patients, and particularly those with more advanced immunodeficiency, are at high risk for active TB after the initiation of ART due to both failure of diagnosis of prevalent TB prior to starting ART, and ongoing persistent immune defects. The diagnosis of “unmasking TB-IRIS” is reserved for a subgroup of patients with ART-associated TB who manifest an acute inflammatory form of TB following ART initiation (see Figure 1 and definitions above) [43].

In the absence of specific diagnostic tests and a robust clinical case definition to distinguish unmasking TB-IRIS from other forms of ART-associated TB, there are limited published data on its clinical manifestations. The reported incidence of unmasking TB-IRIS in South Africa and Uganda ranges between ~1 and 6% [44-47]. In a large prospective study in Kwazulu-Natal, South Africa, unmasking TB-IRIS occurred in 19 out of 498 (3.8%) patients at a median of 12 days (IQR 7 to 49) after initiating ART [45]. This timing is consistent with other studies, where the onset has ranged between 4 to 79 days [44, 48]. The clinical phenotype has been most frequently characterized by lymphadenitis, abscess formation (including in the central nervous system), serositis and pulmonary infiltration [44, 47, 48]. In the Kwazulu-Natal cohort, 3/25 (12%) deaths and 7/65 (11%) hospitalizations were attributed to unmasking TB-
IRIS [45]. Risk factors identified for unmasking TB-IRIS include more advanced immunosuppression, a more pronounced response to ART (greater decline in HIV viral load and larger increase in CD4 cells), intrathoracic adenopathy on pre-ART chest radiograph, anaemia, weight loss, low body mass index, and elevated c-reactive protein [44, 45, 48].

Investigations to confirm the diagnosis of TB should be undertaken in all cases of suspected ART-associated TB, including drug susceptibility testing. It is important to exclude other causes of clinical deterioration on ART, such as other opportunistic infections and malignancies, and adverse drug reactions. There is no specific therapeutic intervention for unmasking TB-IRIS, but continuing ART, providing symptomatic treatments alongside antituberculous therapy and managing complications are key to management [48]. Paradoxical reactions may complicate the clinical course of patients with unmasking TB-IRIS after they have commenced TB treatment [49]. The routine use of corticosteroids is not recommended, but sometimes corticosteroids are used if there are severe inflammatory manifestations. There is no clinical trial evidence to support this strategy.

Despite having similar clinical presentations there is some suggestion that the immunopathology associated with unmasking TB-IRIS may differ from the better-characterized paradoxical form. In one recent study, transcriptomic profiling of TST biopsies from three patients with unmasking TB-IRIS showed increased expression of genes in the Th2 pathway compared to both HIV-negative and -positive controls without TB-IRIS. These findings were supported by increased transcriptional expression and immunostaining of interferon regulatory factor 4 (IRF4), which has been associated with Th2 responses [50]. Similarly to paradoxical TB-IRIS where the primacy of Th1 responses has been questioned,
another study reporting a well-characterised patient with unmasking TB-IRIS showed that tuberculin-specific Th1 responses became expanded only after resolution of IRIS symptoms, and that there was a distorted balance of T-cell phenotypes favouring the central memory T-cell compartment prior to IRIS onset [51]. The role of innate immune effectors, including NK cells, in the pathogenesis of unmasking TB-IRIS has also been highlighted [52]. Additional investigations into the immunological mechanisms are needed, particularly with regard to the role of the innate immune response and the inflammasome, to identify potential predictors and therapeutic targets for unmasking TB-IRIS [9, 53].

**Future directions**

Major insights into TB-IRIS pathogenesis have come from human observational studies with ex-vivo analysis of immune parameters. An appropriate animal model has been lacking, although a mouse model of MAI IRIS has been used [54]. A macaque SIV-Mtb co-infection model using detailed PET-CT imaging to study early immunological changes in the lung is providing useful pathophysiological insights into early HIV-TB co-infection events[55]. This model could in future aid in the study of TB-IRIS, particularly in attributing causality to immune mediators and pathways associated with TB-IRIS in human studies [56]. TB-IRIS pathogenesis studies have identified multiple targets for which biological modulators exist. There is a rationale for moving more candidates to human experimental medicine studies and early stage clinical trials. Even where an interventional study does not show a positive outcome, the pathophysiological insights provided may be extremely valuable. Strategies for future evaluation also include the use of higher doses of corticosteroids for prevention and the use of biomarkers to target preventive and treatment strategies at those most likely to benefit.
**Conclusion**

TB-IRIS causes significant morbidity in resource-limited settings, and mortality risk may be underestimated. A sensitive and specific diagnostic test is lacking, but would be extremely valuable. Biomarkers of risk could aid in identifying high-risk patients for interventions. Clinicians should consider prednisone for TB-IRIS prevention in selected patients. Future research should focus on improving diagnosis and investigating novel therapeutic interventions.

**Key points**

- The incidence of paradoxical TB-IRIS is estimated at 18% (95%CI 16-21%) and may be over 50% in high-risk groups.
- A specific diagnostic test for TB-IRIS is lacking and diagnosis remains a major challenge, especially in low-resource settings.
- Prednisone initiated alongside ART in patients with CD4 ≤100 cells/µl reduced the risk of paradoxical TB-IRIS by 30% in a recent randomised-controlled trial, offering a preventative strategy for selected patients.
- High antigen burden and innate immune mechanisms, including cytotoxic activity, inflammasome activation and matrix metalloproteinases, appear to contribute to TB-IRIS pathogenesis.

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Conflict of Interest Statements

None declared.


Whole blood microarray analysis of tuberculosis meningitis patients demonstrating more abundant neutrophil-associated transcripts prior to TB-IRIS and more abundant transcripts associated with canonical and non-canonical inflammasomes at the time of TB-IRIS onset, in patients who developed TB-IRIS compared to non-IRIS controls.


