Title: Unmasking tuberculosis in the era of antiretroviral treatment

Running title: Unmasked tuberculosis

Authors: Giuseppe Lapadula[1*], Alessandro Soria[1], Alessandra Bandera[1], Nicola Squillace[1], Francesca Sabbatini[1], Fabio Franzetti[2], Giovanni Battista Migliori[3], Andrea Gori[1]

Affiliations:

[1] Clinic of Infectious Diseases, "San Gerardo de' Tintori" Hospital, University of Milano-Bicocca, Monza, Italy

[2] Clinic of Infectious Diseases, "Luigi Sacco" Hospital, University of Milan, Milan, Italy

[3] WHO Collaborating Centre for Tuberculosis and Lung Diseases, Fondazione S. Maugeri, Care and Research Institute, Tradate, Italy

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* Corresponding Author:

Giuseppe Lapadula, MD, PhD

Clinic of Infectious Diseases, "San Gerardo de' Tintori" Hospital, via Pergolesi 33 20052 Monza, Italy

Tel +39 039 233 9320

Fax +39 039 233 9284

Email g.lapadula@hsgerardo.org

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Abstract:

Tuberculosis (TB) can develop soon after antiretroviral treatment initiation, as the result of restoration of anti-TB specific immune response. This form of the disease is often defined as 'unmasked TB', and it represents a major challenge for severely immune-suppressed HIV-infected subjects initiating treatment. Emergence of previously unrecognized TB disease occurs frequently in countries where TB/HIV coinfection is common, and where antiretroviral treatment has become increasingly accessible. The challenges posed by unmasked TB, such as its high incidence, the lack of reliable diagnostic tools and the uncertainties on its optimal management, may hamper our ability to face the TB/HIV epidemic. Therefore, unmasked TB appears a major threat to global health and poses additional barriers to successful HIV/AIDS care and treatment programmes.

This review focuses on the epidemiology, immune-pathogenesis and clinical manifestations of unmasked TB, and provides evidence-based recommendations for management and care of the disease.

Tuberculosis (TB) is the most common opportunistic infection worldwide, and it is a major contributor to the mortality of people living with HIV/AIDS.[1] It is currently estimated that over one million people worldwide have TB/HIV co-infection and the burden of the diseases is particularly high in sub-Saharan Africa. The risk of developing active TB is greatly increased in HIV-infected compared to HIV-negative subjects.[2] Although combination antiretroviral therapy (cART) reduces this risk, TB incidence among treated HIV-infected patients remains higher than that among HIV-negative subjects in the same community.[3, 4]

The rapid expansion of antiretroviral roll-out programmes in high TB burden areas, mainly in southern Africa, has been associated with an unexpectedly high occurrence of TB during the first 3 months of cART, suggesting a potential role of cART-driven immune restoration in 'unmasking' subclinical TB.[3] The underlying mechanisms through which TB is unmasked have yet to be fully explained. A growing body of indirect evidence suggests that the phenomenon is the result of a complex interaction between cART-driven immune restoration and *mycobacterium* antigen load. The resulting disease spectrum may range from typical TB presentations to tuberculosis presenting as an exaggerated inflammatory response due to dysregulated immune restoration, commonly referred to as immune reconstitution inflammatory syndrome (IRIS).[5]

The clinical management of this peculiar manifestation of TB/HIV co-infection can be particularly challenging, due to the difficulties in the diagnosis and the uncertainties on its optimal management. Therefore, unmasked TB appears a major threat to global health and poses additional barriers to successful HIV/AIDS care and treatment programmes. Notwithstanding, to our knowledge no recent review has addressed these topics in major journals.

Aim

Aim of the present perspective article is to highlight the burden of TB unmasked by cART in HIV/TB epidemic and its individual and public health implications, either in low- or high-income settings. We discuss the available evidence on the different aspect of unmasked TB, starting from

the definitions proposed, then analyzing the incidence of TB soon after cART initiation, risk factors and mortality, immune-pathogenesis, clinical features, prevention and treatment.

Methods

The present article is based on a comprehensive review of peer-reviewed studies published on TB unmasked by cART. Literature searches of the PubMed/MEDLINE databases were undertaken to identify pertinent articles. The searches were first performed in September 2009 and then repeated in September 2010 and May 2011, in order to update the reference list. The following research string was used to identify articles focusing on or mentioning unmasked TB: (HIV OR AIDS) AND (TUBERCULOSIS OR TB) AND (UNMASK* OR IRIS OR "IMMUNE RECONSTITUTION"). A second, wider search was conducted in order to identify all studies on TB occurring during antiretroviral treatment. The following search string was used: (HIV OR AIDS) AND (TUBERCULOSIS OR TB) AND (HAART OR ARV OR ANTIRETROVIRAL). All searches were limited to English language. In addition, the websites of the major international congresses on HIV were browsed in order to identify relevant unpublished studies. Conference abstracts older than one year were discarded, if full data were still unpublished.

The articles were then independently sifted by two authors (GL and AB), basing on abstract and, if deemed to be necessary, by reading the full paper. Articles judged to be irrelevant by both authors were discarded, whilst disagreements were resolved through discussion. Eventually, the reference lists of the selected articles were browsed to identify further papers of interest.

Each article was then categorized by its relevance for one or more of the following sub-topics: (i) definition of unmasked TB; (ii) incidence, risk factors and mortality of TB during cART; (iii) immune-pathogenesis; (iv) clinical presentation; (v) prevention and treatment.

Overview of the different definitions of unmasked tuberculosis

Due to lack of diagnostic tests, diagnosis of unmasked TB relies solely on case definition. However, that definition is not standardized, and varies from study to study. Table 1 shows the definitions proposed to date.

During an expert meeting held in Kampala (Uganda) in 2006, a provisional case definition was proposed by the International Network for the Study of HIV-associated IRIS (INSHI).[6] The authors define any new presentation of tuberculosis occurring within the first 3 months of cART as 'cART-associated TB', whilst the term 'unmasking TB-IRIS' is reserved for a subset of cART-associated TB whose clinical course is either characterized by heightened intensity or has been complicated by a paradoxical reaction. 'Unmasking TB-IRIS' is distinguished from TB-IRIS (in the paper termed 'paradoxical tuberculosis-associated IRIS'), defined as a paradoxical reaction and/or a deterioration of an already diagnosed TB, which occurs after the start of cART in a patient receiving anti-TB treatment.

The foremost advantage of this definition is its clinical validity, which has been documented. In a prospective cohort of 498 HIV-infected adults initiating cART in South Africa, the INSHI definition was, in fact, in good agreement with the opinion of an expert panel.[7] Nonetheless, the definition failed to identify about one-third of the cases which had been classified as unmasked TB by the experts. The main reasons for the discrepancy were delayed presentation (more than 3 months after cART initiation), or absence of a 'heightened intensity of clinical manifestation' in cases where, according to expert opinion, immune-reconstitution had played an important role in the clinical presentation of the disease. Criteria regarding the clinical characteristics of the disease can indeed be quite subjective, due to the wide spectrum of TB's manifestations. For this reason, clinical differentiation between incident TB and TB elicited by cART remains difficult in practice.

Other authors refer to 'unmasked TB' as a wide spectrum of TB clinical presentations, triggered by cART-induced immune recovery.[5] In a recent review, Manabe and co-workers proposed a new way of classifying the different forms of TB occurring during cART.[8] According to the authors, 'primary TB' is only temporally associated with cART and is not associated with a rapid recall of

TB antigen-specific memory immune response. Conversely, 'unmasked TB' occurs in the subset of patients who develop clinically recognizable TB after the initiation of cART because of the restoration of TB antigen-specific functional immune responses. Among them, only a proportion of individuals develops an exaggerated inflammatory response, defined as 'unmasked TB-IRIS'. The authors speculate that these three clinical presentations are part of a disease spectrum in which interaction between the TB antigen burden and degree of immune restoration determines the magnitude of clinical symptoms.

Similarly, Lawn and colleagues used the term 'unmasking' TB in reference to subclinical TB that is present but unrecognized before treatment initiation and whose early onset after cART introduction is determined by the restoration of TB-specific immune-response.[9] Both definitions, while theoretically intriguing and biologically plausible, are limited by the absence of objective criteria able to distinguish between pre-existing subclinical TB and incident TB which occurs early during cART as a result of persistent immune-deficiency. The practical utility of such distinction in the clinical practice is, anyhow, questionable.

Haddow and colleagues recently proposed a new general definition of "unmasking IRIS".[10] Its application to TB may resolve some of the problems with previous definitions, by classifying most of the events occurring soon after cART introduction as unmasked TB. According to the authors, the diagnosis of unmasked TB can be made when a strict temporal correlation exists between antiretroviral treatment and TB (i.e., TB presentation within 3 months after initiating cART), even in settings of typical disease progression. Conversely, an atypical or exaggerated course could be considered to be consistent with unmasked TB, even in cases presenting more than 3 months after cART initiation (see Table 1). This comprehensive definition is likely to prove more sensitive than the INSHI definition, but will likely lack in specificity in settings where TB is highly prevalent. Further evaluation in clinical practice is, therefore, required.

As available clinical and immunological markers are as yet unable to differentiate pre-existing from incident TB, a margin of uncertainty and arbitrarity is unavoidable in any definition. Meanwhile, current data support the adoption of the INSHI definition in research practice.

Incidence of tuberculosis and unmasked tuberculosis during combination antiretroviral treatment

Several observational studies have been conducted to assess the burden of TB among individuals receiving cART.[3, 4, 9, 11-32] Estimates of TB incidence, however, differ greatly among the published studies. Differences in TB case definitions, length of follow-up, degree of immune-suppression prior to cART initiation or rate of TB infection in the studied populations are all plausible explanations for the apparent discrepancies. Nonetheless, all the studies consistently report that TB incidence is higher early in the course of cART than at later time points during treatment and, in many cases, more than half of TB cases occurring during cART are diagnosed within the first 3 months of treatment.[11, 13, 23-25, 29, 30, 32] Lawn and colleagues observed that the risk of TB occurrence during the first months of cART, among patients with a CD4+ T-cell counts <200 cells/µl, was 40% (95%CI, 6-61%) higher than among those with the same CD4+ T-cell counts during long-term treatment. The authors suggested that this excess rate of TB is directly attributable to the "unveiling" effect of cART-induced immune restoration.[9] Consistently with this hypothesis, TB rates were reported to remain high, soon after cART initiation and, in some cohorts, even higher than pre-cART rates. In an observational cohort study conducted in USA, incidence of TB during the first 6 months of cART was almost double than that in persons not on cART (0.4 *versus* 0.21 per 100 person-years of follow-up), although the difference was not statistically significant adjusting for CD4+ T-cell count.[17] Similarly, in a South African cohort TB incidence before and within 3 months of cART were 10.5 and 13 per 100 person-years of follow-up, respectively.[32]

Table 2 shows the incidence of TB occurring during cART and shortly after cART initiation in various studies, conducted both in high and low-income settings.

The introduction of cART has dramatically reduced TB incidence among the resident HIV-infected population of high-income countries. The incidence of TB in the Swiss Cohort decreased from 0.78 per 100 person-years before cART to 0.22 per 100 person-years during cART. In this study, the incidence during the first trimester was similar to pre-cART levels, whereas it significantly decreased from month 3 onwards.[11] Two large collaborative cohort studies, conducted in Europe and North America, consistently demonstrated that 0.9-1% of patients beginning cART develop TB during treatment over an extended follow-up and that incidence of unmasked TB during the first months of treatment is 3- to 4-times higher than incidence of TB during later time-points (1.3-1.7 per 100 person-years in the first trimester, compared to 0.3-0.62 per 100 person-years after month 6).[14, 15] This means that, in settings with low prevalence of TB in the general population and high (and early) accessibility of antiretroviral drugs, a significant proportion of TB cases is, in fact, seen in patients receiving cART, with most of these cases occurring soon after cART introduction (up to 15% of all cases of TB in HIV infected individuals in a British case series).[13]

In studies conducted in low-income countries, 3 to 14% of HIV-infected patients developed TB during cART (see Table 2). In these cohorts, the estimated incidence of unmasked TB was 10- to 40-fold higher than in high-income settings, ranging from 5.6 per 100 patient-years [20] to 22.1 per 100 patient-years of follow-up. [22] In other words, whilst in high-income countries, approximately 1 out of every 300 to 1,000 patients is diagnosed with TB within 3 months after starting cART, the proportion of patients diagnosed with unmasked TB in low-income countries ranged from a minimum of 1 case per 80 patients in a South African cohort [3] to a maximum of 1 case per 11 patients in a Cambodian cohort [23], meaning that, unmasked TB, while a clinical and diagnostic challenge worldwide, is a particularly heavy burden in resource-constraint settings.

Risk factors for unmasked tuberculosis upon receipt of antiretroviral treatment

Two factors contribute primarily to the risk of unmasked TB disease after cART initiation: frequency of TB infection in the asymptomatic HIV-infected population and degree of immune-suppression before cART.

In a study comparing TB incidence in 5 different low-income countries, risk of unmasked TB was higher in countries with higher TB burden, such as Senegal and Malawi, than in countries where overall TB incidence was lower, such as Cameroon.[23] In high-income countries, low rates of unmasked TB largely reflect the lower incidence of TB, either in the general population or among HIV-infected subjects, compared to resource-limited countries. Notably, HIV-infected immigrants from countries with high TB incidence may run risks of unmasked TB comparable those run in their country of origin. For instance, in a case-series of 267 individuals immigrated to the United Kingdom from Sub-Saharan Africa, 8 patients (3%) developed TB within 3 months of cART.[13] Similarly, Sub-Saharan country of origin and being foreign born were demonstrated to be risk factors for unmasking TB-IRIS in a French case-control study and in a US observational cohort study, respectively.[16, 17]

The predictive value of baseline CD4+ T-cell count has been convincingly demonstrated in several studies: the lower the CD4+ T-cell count before cART, the higher the risk of TB during cART.[3, 9, 14, 15, 17, 27-29, 32] Lawn and colleagues estimated that the risk of unmasked TB among patients who had a CD4+ T-cell count <200 cells/mm^3 and <100 cells/mm^3 was approximately 3 and 4 times higher than among those with ≥200 CD4+/mm^3, respectively.[9] CD4+ T-cell counts also contribute to the high incidence of unmasked TB recorded in low-income countries, where treatment is generally initiated in the lowest CD4+ strata. One study also suggested that unmasking-TB IRIS is associated with steeper CD4% increases, and more rapid HIV-RNA decline subsequent to cART introduction, compared to non-IRIS-like cART-associated TB.[16]

In developing countries, other factors have been independently associated with increased risk of TB during cART. Most of them, such as low BMI (Body Mass Index), anaemia, World Health Organization (WHO) stage and weight for age, may themselves be indirect measurements of

immune-suppression and disease advancement, independent of CD4+ count.[3, 4, 22, 23, 26, 30] Moreover, low BMI and recent weight loss may also reflect undiagnosed tuberculosis before treatment initiation.[30]

Past history of TB has been linked to risk of recurrence upon cART initiation in two studies conducted in Abidjan (Ivory Coast) and in one conducted in South Africa.[20, 24, 32] However, this association has not been confirmed by other studies and, by contrast, Lawn and colleagues suggested that past treatment for TB, particularly if recently completed, may exert a protective effect against risk of active TB after cART initiation.[3] Given the possible implications in terms of secondary prophylaxis, further studies are needed in order to clarify whether a previous episode of TB poses patients at additional risk of TB disease during cART.

Unmasked TB and risk of death

Unmasked TB is an important cause of mortality and has been associated with a high risk of death. When assessed, the mortality of the patients diagnosed with unmasked TB exceeded 25%.[4, 22, 29] Whilst the occurrence of TB following initiation of cART has been consistently associated with significant higher mortality compared with TB-free patients,[4, 22] it is unclear whether the occurrence of unmasked TB early during the course of cART may pose patients at additional risk of death, compared with TB occurring at later time-points. In one study, the subjects diagnosed with TB within the first 3 months of cART did not experience an excess mortality risk compared with subjects who were diagnosed with TB later.[4] Conversely, in a study conducted in Haiti, patients who received a diagnosis of TB during the first 3 months of cART were 3-times more likely to die than other patients with AIDS and TB.[29] As the authors recognized, in this study, delayed diagnosis and treatment of TB already present at baseline (almost 50% of patients were symptomatic at the time that cART was started) is likely to have greatly contributed to the high mortality. Moreover, despite an attempt to correct for baseline CD4+ T-cell strata, the deepest immune-suppression of patients with unmasked TB may also have influenced their prognosis.

Although it cannot be ruled out that exaggerated response due to unmasking-TB IRIS may also have contributed to high mortality, the overall contribution of TB-IRIS events to TB mortality is generally low. [25, 33]

Anyway, irrespective of its causes, unmasked TB has been associated with high risk of death and, therefore, represents an important burden in low-income countries, leading to a substantial increase of avoidable mortality in antiretroviral roll-out programs.

Immune-pathogenesis of unmasked tuberculosis

Following initiation of antiretroviral treatment, restoration of CD4+ T-cells is documented to occur and is associated with augmentation of bactericidal effector function (cytotoxicity and macrophage-activating cytokine production) with respect to the pre-ART phase.[34] This is reflected in the restoration of a delayed type hypersensitivity reaction to mycobacterial antigens administered intradermally to HIV-infected patients.[35, 36] Several studies have demonstrated that recovery of a protein purified derivative (PPD)-specific response is specific of immune reconstitution with cART.[37-40] Tuberculin-specific T cells are known to recover rapidly, both qualitatively and quantitatively, within 3 to 6 months of cART initiation and successful HIV control. Among HIV/TB coinfected patients, these cells have been shown to reach proportions of up to 33% of the peripheral CD4+ T cells after 9 months of cART and to have an effector memory CD45RA-CD62L+ phenotype. Moreover, a recent study showed that HIV-infected patients with low CD4 T-cell counts ($<200/\mu l$) had a significantly higher proportion of IFN-γ^+ cells after PPD stimulation than either patients with high CD4+ T-cell counts ($>500/\mu l$) or HIV-negative controls. However, analysis of IL-2 and TNF-α production in response to TB antigens, alongside IFN-γ, showed that patients with high CD4+ T-counts had significantly more T-cells co-expressing >1 cytokine than patients with low CD4+ T-cell counts, indicating that a polyfunctional CD4+ T cell profile could be protective against TB in HIV-infection. [41]

Taken together, these findings suggest that the relatively sudden and rapid restoration of functional CD4+ T cells specific for *Mycobacterium tuberculosis* may be massively involved in the pathophysiology of unmasked TB disease after cART initiation. Observations that unmasked TB disease is more common among formerly-naïve patients with very low CD4+ cell counts (<200 cells/µl) and with strong response to cART support this hypothesis.[16]

Expansion of tuberculin-specific T cells has also been cited as a possible cause of IRIS, a complex phenomenon which likely shares a common immunological pathway with unmasked TB. Amplification of interferon-γ (IFN-γ) producing T cells has been reported among patients with TB-IRIS and has been associated with an acute burst of other Th-1 and pro-inflammatory cytokines/chemokines which provokes conditions favouring deregulated immune activation.[42] Similarly, rapid increases in IFN-γ responses to RD1 antigens and to PPD have been found among patients with unmasked TB, suggesting that unmasked TB and TB-IRIS are both associated with restoration of immune response against *M. tuberculosis* antigens.[43] However, increases in IFN-γ responses during TB-IRIS were shown to be relatively slow, compared with unmasked TB, hinting that additional immune defects may play important roles in TB-IRIS. Dysregulation of IFN-γ activity, manifested by higher levels of IL-18 (an inducer of IFN-γ production) and CXCL10 (a marker of IFN-γ production), has indeed been demonstrated during TB-IRIS, but not during unmasked TB.[44] Moreover, circulating IL-10 has been reported to be lower during TB-IRIS events than during non-IRIS events, whereas no differences in IL-10 levels were found between unmasked TB and control events.[45] IL-10 is produced by monocytes and T-regulatory (T-reg) cells and down-regulates many immune functions, including Th1 cytokines and killing of mycobacteria. A defect in T-reg function and a reduction in its ability to downregulate immune response are likely to be among the mechanisms underlying the exaggerated inflammatory response observed during TB-IRIS.[46] In contrast, the role of the T-reg subset would appear to be less important in determining unmasked TB, which is apparently driven by a restoration of effector T-

cell responses, in absence of proper dysregulation between the effector and the regulatory functions of immune response.[45]

Further studies, however, are needed to clarify the role of T-reg cells in the pathogenesis of TB IRIS and unmasked TB. It is likely that exploration of the frequency and the function of these cells, not only in the peripheral blood, but also in the site of disease, could elucidate their exact function.

Figure 1 summarizes the hypothetic immune-pathogenesis of unmasked TB manifesting with (panel A) and without (panel B) IRIS.

Clinical features of unmasked tuberculosis

Unmasked TB can vary widely in the degree of its clinical presentation and there are no clear evidences that unmasked TB is clinically distinguishable from other HIV-associated TB or that unmasking TB has different features from paradoxical TB-IRIS. Restoration of immune function following cART introduction can simply elicit or exacerbate symptoms of TB, or it can be responsible for exaggerated inflammatory response, similarly to TB-IRIS. Table 3 shows the clinical features of unmasked TB cases as reported in published cohort studies, case series and case reports.

Data from larger cohorts show that roughly two thirds of unmasked TB present with lung involvement as primitive and exclusive localization.[22, 23, 31, 32] This contrasts to some extent with data from cohorts of untreated patients with advanced HIV infection, among whom extra-pulmonary involvement accounts for 50% of cases or more.[47, 48] Of note, the high rate of pulmonary TB observed in the first trimester of cART has been associated in particular with a higher incidence of sputum smear negative forms, as compared with TB occurring later during cART.[31, 32] This observation, although potentially influenced by diagnostic capacities, deserves attention, because it can delay diagnosis and treatment of unmasked TB.

Similar diagnostic problems are posed by extrapulmonary TB, which accounted for 30 to 40% of all unmasked TB, in various cohorts. The most common areas of extrapulmonary localization of

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unmasked TB are lymphatic, gastrointestinal and central nervous system.[16, 21, 33, 49-51] Other, less frequent manifestations include glaucomatous hepatitis, genito-urinary TB, splenic abscesses and hypercalcemia. Unusual and extremely severe presentation of unmasked TB have been reported, encompassing adult respiratory distress syndrome [52], bronchiolitis obliterans organizing pneumonia [53], pyomiositis [54], multiple cerebral tuberculomas [50] and haemophagocytic syndrome.[55] These manifestations are likely to be due to an IRIS-like inflammatory reaction, as a consequence of cART-induced restoration of specific-TB immune response.

Prevention of unmasked tuberculosis

Given the high disease burden and mortality of HIV-infected subjects diagnosed with unmasked TB, all possible efforts should be made to prevent it, particularly in developing countries. In the present paper, we will use the term "primary prevention" to indicate interventions aimed at reducing the risk of TB among HIV-infected patients initiating cART. Those interventions aimed at identifying patients at risk of unmasked TB and/or at increasing the yield of early unmasked TB diagnosis will be referred to as "secondary prevention".

Two different, and possibly complementary, strategies are prime candidates for primary prevention: early cART introduction and preventive treatment with isoniazid.

As previously mentioned, unmasked TB is strictly dependent on CD4+ T-cell count: the lower the count, the higher the risk. Timely cART is therefore a potent intervention for unmasked TB prevention, provided that it is initiated before significant immune-depletion. The exact risk reduction associated with different CD4+ T-cell strata, however, is yet to be determined. Whilst it has been convincingly demonstrated that the elevation of the CD4+ threshold for cART eligibility from 200 to 350 cells/μl (or even more) significantly reduces TB among patients awaiting for cART [56], indirect evidences suggest that the benefit of this shift in terms of prevention of unmasking TB is likely to be low. In fact, Lawn and colleagues showed that, among patients with CD4+ T-cell

counts >200/mm^3, TB incidence rates during early cART were comparable to rates during long-term treatment.[9] This suggests that, under this circumstances, the role of immune-recovery in unmasking subclinical TB is less important.

Therefore, in terms of unmasked TB prevention, promoting early HIV diagnosis and minimizing health system delays in cART initiation are likely to be more cost-effective than further anticipating cART debut among patients already on longitudinal care.

Isoniazid preventive treatment (IPT) is a second strategy that could be effective in reducing the risk of developing active tuberculosis and, subsequently, unmasked TB upon cART initiation.

Integration of IPT programs in HIV care programs has been demonstrated to be both feasible and practical, and IPT used concomitantly with cART among the common scenario of patients with advanced HIV infection is likely to have great benefit in terms of preventing unmasked TB. A recent study offered new insights about the benefits of concomitant IPT and cART treatment. Among previously cART-naïve patients with symptomatic disease and/or low CD4+ T-cell counts, a significantly lower mortality rate was observed among those receiving IPT together with cART, compared with those receiving cART alone.[57] Whether this effect is driven, at least in part, by a reduced incidence of unmasked TB is unknown and further study is needed to specifically address the impact of concurrent IPT and cART on unmasked TB among formerly naive patients with advanced HIV disease.

The identification of the patients who are more likely to develop unmasked TB after cART initiation is a mainstay of secondary prevention. Non-specific markers of inflammation, such as D-dimer,[58] C-reactive protein and plasma IFN-γ,[59] have been demonstrated to be higher before cART initiation among those who subsequently develop unmasked TB. Their utility as diagnostic markers is however very limited. Elliott and colleagues proposed that IFN-γ release assays prior to cART might help in predicting unmasked TB and in stratifying patients in low and high-risk groups.[43] Nonetheless, interferon-γ release assays are generally unable to distinguish between active disease and latent infection. Moreover, the need for advanced infrastructure means these tests

are of little or no use in resource-constrained settings. A more interesting marker is lipoarabinomannan (LAM), a major component of *Mycobacterium tuberculosis* cell wall, which can be detected in the urine of TB patients, using a relatively easy and inexpensive procedure. Urine LAM assay was demonstrated to be specific and substantially more sensitive than sputum microscopy as a routine diagnostic TB screening test among patients with CD4+ T-cell counts less than 100 cells/μl.[60]

Treatment of unmasked tuberculosis

Most unmasked TB has a clinical presentation similar to that of incident tuberculosis. Since therapy for susceptible TB is typically as effective in the HIV-infected patient as it is in the general population, prompt initiation of appropriate chemotherapeutic regimen is the only effective therapeutic measure available. Treatment of unmasked TB must be integrated with close follow-up and with immediate revision of cART, in order to minimize adverse interactions between anti-tubercular and antiretroviral drugs, keeping in mind that the use of rifamycins is essential, and that rifabutin is an acceptable alternative to rifampin if this choice is required by drug interaction issues. The response to treatment of unmasked TB is comparable to that of other TB/HIV co-infections, as demonstrated by the low rate of recurrence after TB treatment.[32].

Occasionally, unmasked TB can present with a marked inflammatory component and/or with a clinical course complicated by paradoxical reactions. Management of unmasked TB with a paradoxical presentation has not been extensively studied. However, since the underlying immune-pathogenetic mechanism is likely to be similar to that of TB-IRIS, the therapeutic approach is similar. Published cases and clinical experience suggest that continuation of cART is reasonable in the majority of cases. Paradoxical reactions are self-limiting in most patients, and treatment is usually reserved for severe manifestations, such as life-threatening conditions or in patients at risk of permanent sequelae. Discontinuation of cART should be considered only as a last resort, given

the higher mortality rate of patients with HIV/TB not receiving concomitant antiretroviral treatment.[61, 62] Only in cases "resistant" to corticosteroids, cART interruption should be considered.[63]

In general, symptomatic agents, such as corticosteroids or non-steroidal anti-inflammatory drugs (NSAIDs), are effective in obtaining symptom relief and in controlling inflammatory response. Corticosteroids treatment, although widely used, has yet to be standardized and published studies and case series report varying choices of dosing and treatment duration. Severe IRIS-like reactions can be managed with prednisone at a starting dosage of 1 to 2 mg/kg/day. In cases with central nervous system involvement, dexamethasone use (8 mg twice daily) could be preferred. Steroids were also demonstrated to be safe and effective when used in not life-threatening TB-IRIS. In a recent randomized placebo controlled trial, a 4-weeks course of prednisone reduced hospitalization and hastened the improvement in signs and symptoms associated with TB-IRIS.[64] The risk of adverse reactions attributable to corticosteroids, however, should be carefully evaluated before initiating therapy, because more side effects were reported in the prednisone than in the placebo arm, although the difference did not reach statistical significance.

Occasionally, corticosteroid treatment has been reported to be ineffective in controlling severe cases of TB-IRIS. Where maximal dosages of corticosteroids fail to control a life-threatening inflammatory reaction, alternative drugs can be considered as salvage treatment. Leukotriene antagonists, such as montelukast and zafirlukast, have been considered in a few reports as treatment for IRIS. [65]

Conclusions

All these challenges make unmasked TB a dangerous threat for antiretroviral roll-out and TB control programs, particularly in resource-limited countries, and limit global and national capacities to curb HIV/TB epidemic. Delay in diagnosis and treatment and, in some cases, exaggerated

inflammatory response, are all causes of a high attributable mortality and pose other HIV-infected patients at risk of TB nosocomial transmission. Given the high disease burden, it is crucial to invest all possible efforts to prevent unmasked TB among patients starting antiretroviral treatment, either by impeding TB disease or by increasing the yield of sub-clinical TB diagnosis before treatment initiation. The problem deserves urgent attention by researchers and political commitment, in order to adopt evidence-based pre-emptive policies. Research priorities include the identification of new rapid, affordable and reliable diagnostic tools. Moreover, further investigation on the optimal timing and duration of IPT, as well as implementation of IPT in HIV/AIDS national programs, are urgently needed. Prompt anti-TB treatment and management of paradoxical inflammatory reactions are the cornerstones of unmasked TB treatment.

Statement of interest

None to declare

Legends to figures and tables

Table 1: Unmasked tuberculosis definitions

cART, combination antiretroviral treatment; IRIS, immune reconstitution inflammatory syndrome;

TB, tuberculosis

Table 2: Studies reporting incidence and risk factors for tuberculosis during antiretroviral treatment

Note: data may not directly reflect those reported in the cited papers, because patients with a

diagnosis of TB before cART initiation have been excluded from the table

BMI, body mass index; cART, combination antiretroviral treatment; HIV, human

immunodeficiency virus; IVDU, intra-venous drug user; No., number; TB, tuberculosis

* Data given in square brackets are incidence rates for pulmonary and extrapulmonary tuberculosis,

respectively

§ Data given in square brackets are incidence rates for the first 3 months of antiretroviral treatment,

unless otherwise specified

Table 3. Clinical features of published cohort studies, case series and case reports of unmasked

tuberculosis

ARDS, adult respiratory distress syndrome; cART, combination antiretroviral treatment; CSF,

cerebro-spinal fluid; MDR, multi-drug resistant; No., number; PPD, protein purified derivative; TB,

tuberculosis

Figure 1. Postulated immunopathogenesis of unmasking TB IRIS (panel A) and unmasked TB

without IRIS (panel B).

Both conditions are associated with restoration of immune response against *M. tuberculosis*

antigens due to antiretroviral treatment. Unmasking TB-IRIS (panel A) is characterized by slow

increases in IFN-γ responses, dysregulation of IFN-γ activity, increasing levels of IL-18 and

CXCL10 and defect in T-reg function, leading to exaggerated inflammatory response. Tissue

recruitment of dendritic cells and increase in peripheral γδ T cells contributes to IRIS

manifestations.

Unmasked TB with no IRIS manifestation (panel B) is associated with a more rapid IFN-γ increase

in absence of T-reg functional defects.

Adapted from the Wikimedia Commons files "Image:Macrophage.png " and "Image: Dendritic

cell.png" Source: http://commons.wikimedia.org/wiki

Table 1: Unmasked tuberculosis definitions

Meintjes 2008 [6]	**Unmasking tuberculosis-associated IRIS**
	
	1) Patient is not receiving treatment for TB when cART is initiated
	
	AND
	
	2) Active TB within 3 months of cART initiation
	
	AND
	
	3) Either one of the following:
	
	   a) Heightened intensity of clinical manifestations, particularly if there is evidence of a marked inflammatory component to the presentation.
	
	   OR
	
	   b) Once established on TB treatment, a clinical course that is complicated by a paradoxical reaction.

Manabe 2009 [8]	**Unmasked tuberculosis** 1) Latent TB before the development of HIV-induced immunosuppression AND 2) TB occurring as a consequence of the up-regulation of memory immune response during immune reconstitution induced by cART
Lawn 2009 [9]	**Unmasking tuberculosis** TB presenting during the initial months of immune recovery ("ART-associated TB") whereby immune recovery triggers the presentation of TB.

Haddow 2009 [10]	**Unmasking IRIS**
	
	Clinical criteria
	  1. Temporal relationship: cART initiation must precede clinical deterioration
	  2. New onset of symptoms of an infectious or inflammatory condition after initiation of cART
	  3. Consistent with the presence of pre-existing causative pathogen or antigen at the time of starting cART
	  4. Either of the following
	    a. Onset within 3 months after initiating cART
	    b. Atypical or exaggerated clinical, histological or radiological findings
	
	Exclusion of other causes
	Event not explained by
	  1. Expected clinical course of another condition
	  2. Drug toxicity
	  3. Newly acquired infection
	Failure of antiretroviral treatment

Table 2: Studies reporting incidence and risk factors for tuberculosis during antiretroviral treatment

Study	Location	Population characteristics	No. of patients on cART	No. (%) of TB during cART [Incidence rates]	No. (%) of TB within 3 months of cART [Incidence rates][§]	Early/ Any TB	CD4+ before cART	Factors associated with TB during cART
High-Income Countries								
Ledergerber 1999 [11]	Switzerland	HIV+ starting PI-containing cART	2410	6 (0.2%) [0.22 per 100 person-years]	3 (0.1%) [approx. 0.5 per 100 person-years][§]	50%	Median 188/μl	-
Girardi 2000 [12]	Italy	HIV+ starting cART	387	1 (0.2%)	0	-	-	-
Breen 2005 [13]	United Kingdom	HIV/TB during cART	-	19	16	84%	Median 112/μl	-
Breen 2005 [13]	United Kingdom	Sub-Saharan Africans starting cART	267	11 (4.1%)	8 (3%)	73%	-	-
Girardi 2005 [14]	Europe and North America	Asymptomatic HIV+ starting cART	17142	173 (1%) [0.46 per 100 person-years]	55 (0.3%) [1.3 per 100 person-years][§]	32%	55% <200/μl	cART duration, Calendar year, CD4+ nadir, Risk factor for HIV (IVDU, Heterosexuality)
Brinkhof 2007 [15]	Europe and North America	HIV+ starting cART observed over 1 year	22217	205 (0.9%) [1 per 100 person-years]	[1.7 per 100 person-years][§]	-	Median 234/μl	CD4+ nadir, Risk factor for HIV (Heterosexuality)
Valin 2010 [16]	France	HIV+ starting cART	-	11 unmasking TB-IRIS	-	-	Median 116/μl	Sub-Saharan African, baseline HIV-RNA, CD4% increase, HIV-RNA decline
Pettit 2011 [17]	USA	HIV+ starting cART	3492	17 (0.5%) [0.13 per 100 person-years]	7 (0.2%) [0.4 per 100 person-years within 6 months of cART][§]	41%	Median 242/μl	Male gender, black ethnicity, Place of birth (foreign born), Risk factor for HIV (Heterosexuality), CD4+ nadir, baseline HIV-RNA
Low-Income Countries								
Sungkanuparph 2003 [18]	Thailand	AIDS patients starting cART	60	8 (13.3%)	-	-	Median 9/μl	-
John 2005 [19]	Uganda	HIV/TB during cART	-	131	29	22%	-	-
Seyler 2005 [20]	Ivory Coast	HIV+ starting cART	129	12 (9.3%) [4.8 per 100 person-years]	3 (2.3%)	25%	Median 129/μl	Previous TB diagnosis
Lawn 2005 [3]	South Africa	HIV+ starting cART	346	27 (7.8%) [2.4 per 100 person-years]	4 (1.2%) [3.35 per 100 person-years within 1 year of	15%	Median 242/μl	Age, CD4+ nadir, WHO stage.

|						cART][§]		
Baalwa 2005 [21]	Uganda	HIV+ without TB starting cART	271	26 (9.6%)	8 (2.8%)	31%	-	-
Lawn 2006 [22]	South Africa	HIV+ without TB starting cART	756	81 (10.7%) [10.5 per 100 person-years]	40 (5.3%) [22.1 per 100 person-years][§]	49%	Median 96/μl	Current CD4+
Bonnet 2006 [23]	Cambodia	HIV+ starting cART	717	100 (13.9%) [7.6; 12.7 per 100 person-years]*	63 (8.8%)	63%	66% <50/μl	-
Bonnet 2006 [23]	Thailand	HIV+ starting cART	500	57 (11.4%) [10.4; 4.3 per 100 person-years]*	30 (6%)	53%	72% <50/μl	-
Bonnet 2006 [23]	Kenya	HIV+ starting cART	654	73 (11.2%) [17.6; 6.9 per 100 person-years]*	41 (6.3%)	56%	36% <50/μl	-
Bonnet 2006 [23]	Malawi	HIV+ starting cART	1064	80 (7.5%) [14.3; 2.1 per 100 person-years]*	50 (4.7%)	62%	21% <50/μl	-
Bonnet 2006 [23]	Cameroon	HIV+ starting cART	216	10 (4.6%) [4.8; 0 per 100 person-years]*	8 (3.7%)	80%	25% <50/μl	-
Brinkhof 2007 [15]	Africa, Asia and South America	HIV+ starting cART	4540	258 (5.7%) [7.4 per 100 person-years]	[10.7 per 100 person-years][§]	-	Median 107/μl	CD4+ nadir, gender, age
Moore 2007 [4]	Uganda	HIV+ without TB starting cART	969	53 (5.5%) [3.9 per 100 person-years]	23 (2.4%) [7.5 per 100 person-years within 6 months of cART][§]	43%	Median 127/μl	Low BMI
Moh 2007 [24]	Ivory Coast	HIV+ without TB starting cART	792	23 (2.9%) [3.3 per 100 person-years][§]	[5.6 per 100 person-years][§]	~50%	Median 252/μl	Previous TB diagnosis, haemoglobin, current CD4+
Murdoch 2008 [25]	South Africa	HIV+ without TB starting cART	338	16 (4.7%)	11 (3.2%)	69%	Median 115/μl	-
Huruy 2008 [26]	Ethiopia	HIV+ without TB starting cART	178	20 (11.2%)	-	-	Mean 123/μl	
Etard 2009 [27]	Senegal	HIV+ without TB starting cART	352	42 (11.9%) [2.3 per 100 person-years][§]	[4.5 per 100 person-years within 1 year of cART][§]	-	Median 128/μl	Haemoglobin, CD4+ nadir
Smith 2009 [28]	South Africa	Children <2 years old starting cART	169	12 (7.1%)	11 (6.5%)	92%	39% <15 CD4%	Age <6 months, CD4%, HIV-RNA, weight for age
Koenig 2009	Haiti	HIV/TB during cART	-	97	49	50.5%	-	CD4+

[29]								
Bedell 2009 [30]	Malawi	HIV+ without TB starting cART	2281	-	67 (2.9%)	-	-	Weight loss >10%, WHO clinical stage 4
Lawn 2009 [9]	South Africa	HIV+ without TB starting cART	1032	171 (16.6%) [7.3 per 100 person-years	[18.8 per 100 person-years]§	-	Median 97/μl	Current CD4+
Dembelé 2010 [31]	Burkina Faso	HIV+ starting cART	2383	70 (2.9%)	33 (1.4%) [5.77 per 100 person-years]§	47.1%	Mean 128/μl	Low BMI, CD4+ nadir
Nicholas 2011 [32]	South Africa	HIV+ without TB starting cART	19325	933 (4.8%) [5.4 per 100 person-years	470 (2.4%) [13 per 100 person-years]§	50.4%	Median 142/μl	Urban sites, male gender, calendar year, low BMI, previous TB diagnosis, CD4+ nadir

Table 3: Clinical features of published cohort studies, case series and case reports of unmasked tuberculosis.

Study	Location	No. of cases	Symptoms before cART initiation	Clinical features	Sputum smear/culture
Shelburne 2002 [49]	USA	1	Not reported	Fever, weight loss, abdominal pain due to bowel (cecal) tuberculosis.	Culture-positive
Goldsack 2003 [52]	UK	1	Asymptomatic. Normal chest x-ray	Fever, night sweats, shortness of breath rapidly evolving in respiratory failure due to ARDS	Smear- and culture-positive
John 2005 [19]	Uganda	1	Malaise, weight loss, low-grade fever, intermittent cough. Normal chest x-ray	Fever, dry cough, miliary pulmonary infiltrates.	Smear- and culture-negative
Seyler 2005 [20]	Sub-Saharan Africa	3	Not reported	1 pulmonary, 2 disseminated	2 smear-positive culture-positive
Breen 2005 [13]	England	13	60% symptomatic (5/13 fever, 4 weight loss, 2 cough, 2 night sweats)	38% pulmonary 24% extrapulmonary 38% miliary 61% 'paradoxical reactions'	85% culture-positive
Miller 2006 [66]	UK	1	Weight loss, minimal productive cough, fever	Pulmonary TB	Smear- and culture-negative
Bonnet 2006 [23]	Cambodia, Thailand, Kenya, Malawi, Cameroon	192	Not reported	68% pulmonary 32% extrapulmonary	Not reported
Lawn 2006 [22]	Sub-Saharan Africa	40	Not reported	78% pulmonary 22% extrapulmonary	41% smear-positive 81% culture-positive
Zampoli 2007 [67]	South Africa	7 children	Not reported	6 pulmonary TB 1 disseminate TB (pulmonary+lymphoadenitis+meningitis)	4/6 Smear- and culture-positive (1 MDR) 1/6 Smear-negative and culture-positive 2/6 Smear- and culture-negative
Park 2007 [51]	South Korea	5	Not reported	3 pulmonary, 2 extrapulmonary (1 cerebral) Lymphadenitis in 3/5 cases	Not reported
Moh 2007 [24]	Ivory Coast	23	Not reported	64% pulmonary 24% extrapulmonary 12% disseminated	Not reported
Dautremer 2007 [50]	France	1	Not reported	Tuberculous meningitis (acute onset), multiple cerebral tuberculomas	Culture-positive CSF
Tuon 2007 [68]	Brazil	1	Not reported	Hepatosplenic abscesses and	Culture of liver biopsy negative

				granulomatous hepatitis	
Meintjes 2008 [6]	Not reported	1	Low-grade fever, retrosternal chest pain, dry cough. Normal chest X-ray	Fever and respiratory distress due to pulmonary tuberculosis	Smear-positive
Ocama 2008 [69]	Uganda	7	Not reported	Suspected TB hepatitis	Not reported
Baalwa 2008 [21]	Uganda	8	Not reported	1 pulmonary, 7 extrapulmonary (2 meningitis, 1 lymphadenitis, 1 abdominal), 3 disseminated	1 smear-positive
Murdoch 2008 [25]	South Africa	18	Not reported	50% pulmonary, 25% extrapulmonary, 25% disseminated	69% smear- or culture-positive
Lawn 2009 [53]	South Africa	1	Asymptomatic	Bronchiolitis obliterans organizing pneumonia, death	Smear- negative
Lawn 2009 [9]	South Africa	203	Not reported	74% pulmonary, 26% extrapulmonary	Not reported
Manabe 2009 [8]	USA	1	Chronic watery diarrhoea, subjective fevers, chills, weight loss. Normal Chest X-ray. PPD negative.	Fever and cough due to upper lobe cavitary tuberculosis	Smear-negative, culture-positive
Castelnuovo 2009 [33]	Uganda	2	Asymptomatic	1 extrapulmonary TB, 1 TB meningitis	Not reported
Tsao 2009 [70]	Taiwan	1	Weight loss	Hypercalcemia, splenic abscess	Not reported
Chen 2009 [54]	Taiwan	1	Weight loss	Gluteal pyomiositis	Not reported
Soria 2009 [55]	Italy	1	Not reported	Haemophagocytic syndrome due to disseminated TB	Smear- and culture-negative
Valin 2010 [16]	France	11	Not reported	4 pulmonary, 3 extrapulmonary, 4 disseminated (2 CNS involvement)	All culture-confirmed
Pettit 2011 [17]	USA	7	Not reported	2 not reported, 5 extrapulmonary IRIS (2 lymphadenitis, 1 splenic, 2 disseminated)	Not reported

Figure 1. Postulated immunopathogenesis of unmasking TB IRIS (panel A) and unmasked TB without IRIS (panel B).
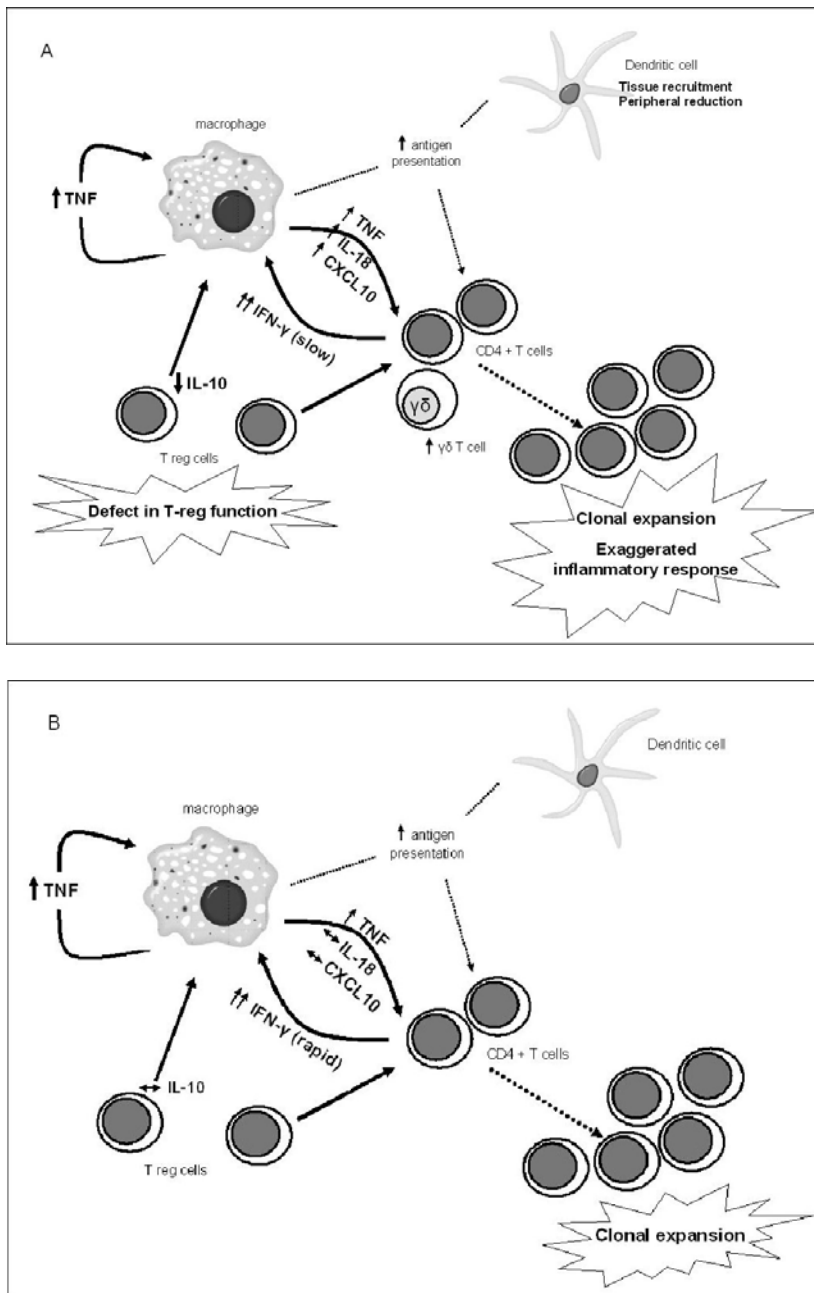
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