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# Tuberculosis and HIV disease: Two decades of a dual epidemic

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**Summary.** The HIV epidemic is currently in its third decade without any sign of abating. Tuberculosis (TB) is responsible for a third of all AIDS deaths, 99% of which occur in developing countries. The two epidemics fuel each other, together making up the leading infectious causes of mortality worldwide. Tuberculosis- HIV coinfection presents special diagnostic and therapeutic challenges and constitutes an immense burden on the health care systems of heavily infected countries. Despite major gains that have been made in the past two decades, important questions still remain. To cope with the challenge of TB-HIV coinfection, further research in the design of diagnostic tests for tuberculosis, detection of drug resistant *Mycobacterium tuberculosis* strains in HIV-positive people, as well as development of more effective therapeutic agents and vaccines are urgently needed. It has become evident that this dual epidemic will persist unless comprehensive measures are instituted through the provision of sufficient funding in addition to expanding and strengthening current control strategies adopted by governments and international organizations.

**Key words:** Tuberculosis, HIV, coinfection, review.

## Introduction

Tuberculosis remains the leading infectious disease in the world with approximately 90 million incident cases occurring during the decade 1990 through 1999 [1]. Tuberculosis is also responsible for 6% of all deaths worldwide, and is the world's foremost cause of death from a single infectious agent in adults [1]. It is estimated that about one third of the world's population have been infected with *Mycobacterium tuberculosis*, the overwhelming majority of whom reside in developing countries [2].

In the United States, from 1953 through 1984, the incidence of tuberculosis disease declined an average of 5% annually. From 1985 through 1992, there was a 20% increase in total cases of tuberculosis in the US (Fig. 1), and a 40% increase in tuberculosis cases among children [3].

Four major factors have been advanced as explanations for the upsurge:

(1) The co-epidemic of HIV infection: HIV is the strongest risk factor for the development of tuberculosis disease [4]. In addition, tuberculosis is the commonest opportunistic infection in HIV-infected individuals [5].
(2) The increase in immigration of people to the United States from countries with a high prevalence of tuberculosis, thus expanding the pool of infected persons [6]. This is, however, controversial as some excellent studies have shown that recent transmission of identical strains of the bacilli is more frequently found among US-born individuals compared to reactivation of latent infection by unique strains that are predominant in foreign-born patients [7], a situation that will make US-born individuals more likely to be the source of recently acquired TB disease.
(3) Increased transmission of *M. tuberculosis* in congregate settings, such as jails, prisons [8], hospitals [9], nursing homes, and homeless shelters [10].
(4) General decline in tuberculosis-related public health services and access to medical care for the indigent in many communities [11].

As a result of enhanced public health interventions, increased funding for tuberculosis control programs, introduction of directly observed therapy (DOT), and prevention measures to curb nosocomial transmission of tuberculosis, the incidence of the disease in the US declined 26% between 1992 and 1997 [12–14]. Despite these encouraging results, the rate of decline of TB falls far short of the targeted rate of 3.5 per 100,000 by the year 2000 if the goal of a rate of $< 1$ case per 100,000 is to be achieved by the year 2010. As of 1998, about 19,000 incident cases occurred nationwide, a confirmation that tuberculosis still remains a public health problem deserving more attention and public commitment (Fig. 1).

## Tuberculosis and human immunodeficiency virus (HIV) infection: Symbiotic interaction

Tuberculosis is the most common opportunistic infection in individuals diagnosed with the human immunodeficiency virus [5]. In HIV-infected persons, early progression of newly acquired tuberculosis infection may occur in almost 40% of persons within 4 months, compared with 2–5% of historical controls in the first 2 years [15]. Among persons already infected with tuberculosis, superimposed co-infection with HIV leads to the develop-

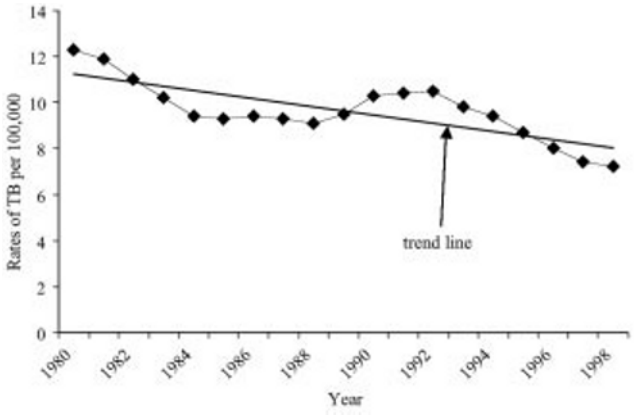


**Fig. 1.** Rates of tuberculosis in the United States, 1980–98

ment of active tuberculosis at an annual rate of 7–10%, compared with a lifetime risk of reactivation of 5–10% [16]. In 1993, the US National Program for Tuberculosis Surveillance was expanded, and additional variables, including HIV sero-status, were added to the RVCT (Report of Verified Case of Tuberculosis), a data collection form developed by the Centers for Disease Control and Prevention (CDC) that serves as the main instrument for information retrieval from TB patients throughout the United States. The analysis of national TB surveillance by HIV status is unfortunately hampered by the lack of complete information. The proportion of TB individuals in the United States with known HIV status between 1993 and 1998 is illustrated in Fig. 2. The graph shows a low level of HIV testing in tuberculosis cases in the US ranging from 33% in 1993 to 55% achieved in 1998. This low level of HIV testing among TB patients is a reflection of region or state-specific practice. A study conducted in Los Angeles found that nearly 40% of all patients with TB did not have testing performed for HIV and those that did usually reported risk factors for HIV [17]. Estimates for TB-HIV coinfection from such data are therefore, likely to be fraught with errors.

To improve such estimates, state health departments have compared TB and AIDS registries to determine, to an acceptable degree of precision, the proportion of TB cases coinfected with HIV. Also, multi-center surveys in tuberculosis clinics as well as extrapolation estimates based on pooled data of national TB estimates have been conducted in order to arrive at unbiased figures. In Table 1, a summary of such studies carried out in the US is given. Only those studies that have large sample size of at least 1000 TB cases are included. The level of coinfection in the studies varied from 5.8–38.9%. However, this estimate is by no means a measure of the risk for tuberculosis in already HIV-infected individuals. The incidence of tuberculosis in HIV-seropositive persons varies from 0.7% per year to 9% per year [18–22]. Determining such a risk is obviously not easy. Firstly, detected TB incident cases will largely depend on the length of period of observation of the HIV-cohort. Secondly, the extent of immunosuppression as well as the level of the prevalence of other risk factors for tuberculosis in a given population under study could account for substantial variations in estimates across studies.

The risk of tuberculosis among HIV-infected individuals is correlated to socio-demographic factors and the degree of immunosuppression of the patient. In the United States, eastern location has been reported as a very strong demographic risk factor [18]. This may be related to the clustering of active cases of tuberculosis in these areas, a fact that enhances exposure probability. Male gender, being US-born (as compared to foreign-born), and falling within the age range 25–44 are characteristics that elevate the likelihood of tuberculosis among HIV patients [18, 23]. Registry matched comparison revealed that about 14% of all TB cases were coinfected in the total population compared to 27% in the age group 25–44 years [13]. The frequency of coinfection is four times higher in males than in females, and about seven times in US-born as compared to foreign-born individuals [23]. In another population-based investigation, it was found that up to 71% of co-incident cases occurred in non-Hispanic blacks [24]. A CD4 count of less than 200 cells/mm$^3$ and non-reactivity to mumps antigen (independent of purified protein derivative response) are indicators of increased risk for tuberculosis among HIV-positive persons [18].

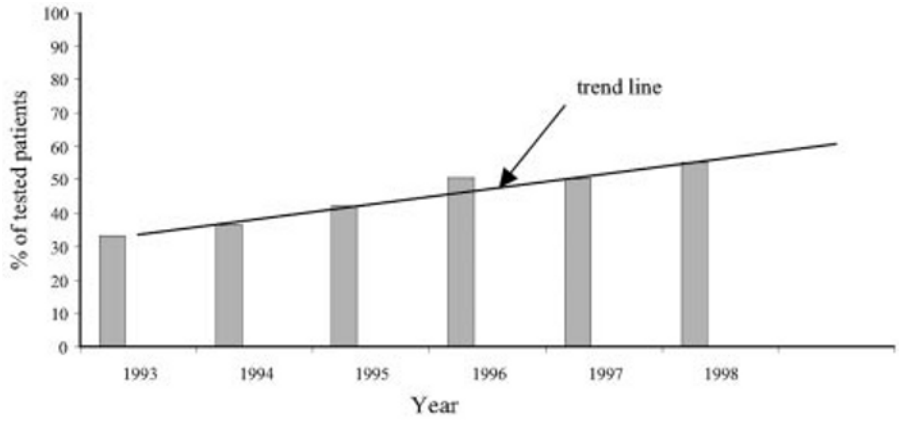


**Fig. 2.** Level of HIV testing among patients diagnosed with TB in the United States, 1993–98

**Table 1.** Level of HIV-TB coinfection in the United States

| Study | No. of TB cases | No. (%) of coinfected patients | Type of study |
|---|---|---|---|
| Onorato et al. (1993) [25] | 9524 | 2000 (21) | Multi-center urban clinics |
| Mueller et al. (1995) [24] | 3738 | 458 (12) | Population-based (Chicago) |
| Snyder et al. (1997) [23] | 1990 | 116 (6) | Population-based (Alameda County, CA) |
| Markowitz et al. (1997) [18] | 26673 | 5840–8760 (22–33) | Extrapolated estimate based on 1992 US national data on TB |
| Markowitz et al. (1997) [18] | 22813 | 5840–8760 (26–38) | Extrapolated estimate based on 1995 US national data on TB |
| Sotir et al. (1999) [26] | 1378 | 536 (39) | Hospital-based (Atlanta) |
| Aliyu et al. (2003) [88] | 1065 | 220 (21) | Retrospective cohort study (North Carolina) |

## Vulnerability of HIV-infected individuals to tuberculosis: Immunological basis

### *Theory of cellular immune response*

It was suggested as far back as the 1940's that cell-mediated immune response was the main immune defense mechanism in containing tuberculosis infection [27]. Lurie's pioneering investigation at the University of Pennsylvania involved two groups of animals infected with tuberculosis bacilli. In one arm of the study were naïve, normal animals not previously exposed to TB organisms, while in the other arm were "immune" animals which had previously experienced active tuberculosis. In order to evaluate the influence of macrophages from the two sources on the replication of tubercle bacilli, he explanted lymph node cells into the anterior chamber of a normal rabbit eye that served as a culture medium. By quantifying mycobacterial growth colonies, he came to the following important conclusions, which formed the basis for the theory of cellular immune response to tuberculosis infection:

(1) Mononuclear phagocytes of immunized animals that had ingested tubercle bacilli in vivo and had subsequently been transplanted and allowed to grow in a milieu of a normal naïve animal continued to inhibit the multiplication of the microorganism.
(2) Mononuclear phagocytes of immunized animals that had ingested tubercle bacilli in vitro in the presence of immune serum inhibit the multiplication of the microorganism in their cytoplasm to a much greater extent than cells of normal animals that had ingested the bacteria in the same medium and had grown in a similar environment.

About two decades later, Mackaness [28] showed that the immune response of mice to infection with *Listeria monocytogenes* gave rise to a population of immunologically committed lymphoid cells which had the capacity to confer protection and a proportionate level of delayed hypersensitivity upon normal recipients. He also inferred that the anti-bacterial resistance conferred by immune lymphoid cells was not due to anti-bacterial antibody, but was mediated indirectly through the macrophages of the recipient. These became activated by a process that appeared to depend upon some form of specific interaction between the immune lymphoid cells and the infecting organism. This established the concept of a lymphocyte-macrophage interaction, the exact nature and mechanism of which were determined by subsequent workers [29].

### *Mechanism of cellular immune response to Mycobacterium tuberculosis*

Mycobacterial antigens in the host peripheral circulation are recognized by cells expressing the CD4 or T4 epitope, a process that is restricted by Class II molecules of the major histocompatibility locus. This results in cellular activation, proliferation and production of cytokines. These substances serve as mediators and induce macrophages to inhibit the replication of certain mycobacterial strains [30].

Susceptibility to tuberculosis is related to the type of cytokines produced by T-lymphocytes. Interferon-γ (IFN-γ) has been identified as the central cytokine that modulates the induction of macrophages, and fatal mycobacterial disease develops in children who lack the Interferon-γ receptor [31].

Activation of macrophages following exposure to IFN-γ is partially mediated by 1,25-dihydrovitamin $D_3$, a form of vitamin $D_3$ that has been shown to induce a state of tuberculostasis in monocytes/macrophages [32]. This is apparently the scientific explanation for the historical observation of the beneficial effects of sunlight in the care of tuberculosis patients.

Immunologic response targeted against mycobacteria could occur via direct killing of the organism by T-lymphocytes that express the CD8 or T8 epitope (Table 2). Cells of this type respond to the entry of foreign antigens through linkage with the Class I molecules of the major histocompatibility complex. T8 lymphocytes are capable of directly lysing target cells expressing mycobacterial antigens, and could also, to some extent, augment target cell killing of mycobacteria via production of cytokines [29].

**Table 2.** Type of lymphocyte response to *Mycobacterium tuberculosis*

| | Proliferation | Cytokine secretion | Target cell lysis |
|---|---|---|---|
| CD4+ | ++++ | +++ | – |
| CD8+ | ++++ | + | ++++ |
| CD4-8- | ++++ | +++ | – |

There is also evidence for the existence of another sub-population of T-lymphocytes which function independently of the histocompatibility locus. These lymphocytes do not express T4 or T8 epitope, instead, they are characterized by the composition of the T-cell receptor, which in this case is of the gamma/delta type [33].

### *The role of macrophage effector cells*

Macrophages are the main effector cells that directly kill phagocytosed mycobacteria. They constitute cellular reservoir for mycobacteria which are often found residing within intra-cytoplasmic phagocytic vesicles. Macrophages generate oxygen radicals which are highly toxic to ingested micro-organisms, although this mechanism may not necesssarily apply to mycobacteria [34]. It is possible that other pathways of microbacterial killing play key roles, such as the arginine-dependent mechanism for the production of nitrous oxide radicals.

### *Enhancement of mycobacterial replication in HIV setting*

When peripheral blood lymphocytes from HIV-infected persons with tuberculosis are exposed to *Mycobacterium tuberculosis* in vitro, they produce less interferon-γ but similar amounts of interleukin-4 and interleukin-10, as compared to lymphocytes from HIV-negative patients with tuberculosis [35]. These findings suggest that the reduced T1 response in HIV-infected patients contributes to their susceptibility to tuberculosis [36].

Many of the macrophage effector functions are depressed in HIV-infected persons. For example, receptor-mediated phagocytosis and oxidative activity are impaired [37]. Chemotaxis – the first step in the recognition and killing of microbial pathogens – is also deficient for a variety of migration signals [38]. These alterations that affect macrophage function may be attributable to the component parts of HIV present in the body's circulatory system or the extracellular milieu around an infected cell. The glycoprotein coat of HIV (gp 120), for instance, is capable of changing macrophage effector function independently of the whole retrovirion by altering the expression of macrophage receptors for chemotactic ligands [39].

### *Enhancement of HIV replication in tuberculosis-infected environment*

Tuberculosis provokes a more severe prognosis in HIV-infected persons than that observed in sero-negative subjects [40–42]. It has also been demonstrated that TB contributes to the progression of HIV disease [43], and markedly elevated HIV plasma load correlates to active tuberculosis [44]. *Mycobacterium tuberculosis* and its cell wall component *lipoarabinomannan* (LAM) increase the release of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) by mononuclear phagocytes at the level of transcription by activation of promoters through increased activity of DNA binding proteins such as nuclear factor- (NF)-αβ and NF-IL6 [45, 46]. The HIV-1 long terminal repeat (LTR), like the promoters of several cytokine genes, contains NF-κβ and NF-IL6 binding sequences. TNF-α is said to increase HIV-1 production in mononuclear phagocytes through transcriptional activation of the LTR promoter by NF-κβ [47], although the effect is partially dependent on the state of cellular differentiation [48–50]. Similarly, *M. tuberculosis* and its cell wall component LAM increase HIV-1 promoter activity, increase binding of NF-κβ to LTR sequences, and enhance HIV-1 replication in cultured cells [51–53].

In another recent study [54], it was demonstrated that IL-6 and TNF-α present in tuberculosis-infected fluids supported HIV replication. Furthermore, in contrast to the usual requirement of in vitro pre-activation of peripheral blood lymphocytes (PBL) to ensure their productive infection by HIV, pleural lymphocytes from TB patients could be directly infected without any prior in vitro activation and could support a constant viral replication [54].

These complex mechanismal pathways could be summarized as follows:

- Exposure of alveolar macrophages and lymphocytes from HIV-infected individuals to *M. tuberculosis* in vitro up-regulates retroviral replication [44, 55].
- In HIV-infected patients with pulmonary tuberculosis, the concentration of retroviral RNA in bronchoalveolar-lavage fluid is highest in areas of tuberculosis involvement.
- Pleural fluid from patients with tuberculosis increases HIV replication in activated lymphocytes.
- *M. tuberculosis* enhances HIV replication by inducing macrophages to produce tumor necrosis factor-α, interleukin-1, and interleukin-6. The microenvironment generated by TB supports a productive HIV infection of lymphocytes through the local production of enhancing cytokines, namely, TNF-α and IL-6.

## Clinical features and radiologic findings in coinfection

There is now evidence that clinical presentation of tuberculosis differs according to HIV-status. Extrapulmonary foci of tuberculosis are observed more frequently in HIV-positive than in HIV-negative patients, and this becomes more pronounced as the degree of immunodepression increases in HIV-infected individuals [56]. Since cellular immune response is central in containing mycobacterial infection, it has been suggested that the manifestations of tuberculosis depend on the stage of HIV-induced immune-incompetence [40, 57–60]. In tuberculosis patients that are HIV-coinfected but without evidence of immunodeficiency, extrapulmonary TB was uncommon, chest x-rays usually showed features suggestive of reactivation tuberculosis, and tuberculin skin tests were usually positive [61, 62].

A positive skin test is more common in tuberculosis patients with less severe immunodeficiency [63, 64]. Us-

ing a cut-off point of ≥5 mm induration to a challenge of purified protein derivative (PPD) has been found to be a moderately sensitive test for tuberculosis in patients with >100 CD4 cells/μL. These findings could be explained by the CD4 lymphopenia characteristic of advanced stages of HIV infection. CD4 cells that produce interleukin-2 (IL-2) and interferon-γ (IFN-γ) are thought to be critical to development of a positive tuberculin skin test [65]. Their depletion in advanced HIV disease explains frequent negative tuberculin test and anergic response in affected patients with coinfection.

The clinical presentation of abdominal tuberculosis in HIV-infected patients is characterized by visceral lesions and intra-abdominal lymphadenopathy with necrosis, best visualized by computed tomography. In contrast, ascites and omental thickening are characteristic findings of abdominal TB in HIV-negative persons [66]. The clinical presentation of tuberculous meningitis is said to be similar in both populations, except that intracerebral mass lesions (e.g. tuberculoma) are more frequent in HIV-infected patients [67]. Mycobacteremia and positive acid-fast smears have been found to correlate inversely with levels of CD4 cell counts [56].

Atypical radiographic changes of pulmonary tuberculosis have been reported in HIV-infected patients [61, 68–70]. As a result, suspicious diagnosis based on chest X-ray examination and consequently, the commencement of anti-tuberculous therapy may be delayed in these patients. The manifestations of TB in HIV-infected persons have also been noted to vary by the level of immunosuppression [71–73]. In addition, the radiologic manifestations of primary and reactivated TB differ [68–70], and as many as 30% of TB cases may be attributable to primary TB in areas with high HIV prevalence [74, 75]. It is likely that a considerable number of the atypical radiologic features of HIV- related TB is due to this greater proportion of primary TB among HIV-coinfected individuals [76]. The association of certain radiographic changes with the extent of HIV-related immunosuppression, as reflected by CD4+ cell counts, may be the product of different pathogenic mechanisms of TB [77]. Table 3 summarizes the results of a pooled analysis of studies that compare degree of immunosuppression and radiologic findings in coinfected patients.

Hilar or mediastinal adenopathy is more common among those with HIV-related TB than among HIV-uninfected persons with TB, and among those with HIV infection, adenopathy is more common in patients with advanced immuno-suppression (Table 3). The association between advanced immunosuppression, using low CD4+

**Table 3.** Pooled analysis of chest radiographic findings, as related to CD4+ count (/mm$^3$)/AIDS status

| Radiographic finding, Study | No. (%) of patients in indicated category | | Odds ratio | P-value* |
|---|---|---|---|---|
| | CD4+ count <200 (AIDS) | CD4+ count >200 (No AIDS) | | |
| Cavitation | | | | |
| Mukadi et al. (1993) [78] | 48 (56) | 91 (79) | 0.3 | <0.001 |
| Batungwanayo et al. (1992) [71] | 35 (29) | 13 (69) | 0.2 | 0.02 |
| Pastores et al. (1993) [79] | 16 (6) | 6 (17) | 0.3 | 0.48 |
| Keiper et al. (1995) [80] | 26 (15) | 9 (67) | 0.1 | <0.001 |
| Perlman et al. (1997) [77] | 98 (7) | 30 (20) | 0.3 | 0.08 |
| Pooled analysis [95% CI] | | | 0.3 [0.16–0.44] | <0.001 |
| Adenopathy | | | | |
| Jones et al. (1993) [56] | 58 (36) | 30 (13) | 3.7 | 0.3 |
| Batungwanayo et al. (1992) [71] | 35 (40) | 13 (8) | 8.0 | 0.04 |
| Pastores et al. (1993) [79] | 19 (100) | 6 (100) | –** | –** |
| Keiper et al. (1995) [80] | 26 (23) | 9 (11) | 2.4 | 0.65 |
| Perlman et al. (1997) [77] | 98 (30) | 30 (7) | 5.9 | 0.01 |
| Pooled analysis [95% CI] | | | 4.6 [2.19–9.74] | <0.001 |
| Pleural effusion | | | | |
| Jones et al. (1993) [56] | 58 (10) | 30 (27) | 0.3 | 0.07 |
| Batungwanayo et al. (1992) [71] | 35 (43) | 13 (46) | 0.9 | 1.00 |
| Pastores et al. (1993) [79] | 19 (21) | 6 (33) | 0.5 | 0.61 |
| Keiper et al. (1995) [80] | 26 (15) | 9 (11) | 1.5 | 1.00 |
| Perlman et al. (1997) [77] | 98 (7) | 30 (10) | 0.7 | 0.70 |
| Pooled analysis [95% CI] | | | 0.6 [0.31–1.15] | 0.12 |
| Infiltrates | | | | |
| Mukadi et al. (1993) [78] | 48 (88) | 91 (96) | 0.3 | 0.09 |
| Batungwanayo et al. (1992) [71] | 35 (94) | 13 (100) | –** | 1.00 |
| Perlman et al. (1997) [77] | 98 (52) | 30 (67) | 0.5 | 0.21 |
| Pooled analysis (95% CI) | | | 0.5 [0.22–0.90] | 0.02 |

* *P* values are for Fisher's exact test (two-sided), except for the pooled analyses, where *P* values are for the Mantel-Haenszel $\chi^2$ test. ** Could not be computed because of zero-containing cells.

count as marker, and intrathoracic lymphadenopathy has been demonstrated to be independent of opportunistic processes, such as MAC (*Mycobacterium avium complex*), lymphoma, histoplasmosis or Kaposi's sarcoma [77].

The presence of cavities in the lung parenchyma of tuberculosis patients correlates with delayed-type hypersensitivity response, and is usually a manifestation of reactivated TB [73]. Cavitations are found more commonly in HIV-negative patients or those HIV-infected individuals with CD4+ counts $\geq 200$ cells/μL and in those with less advanced HIV infection (Table 3). This suggests that radiographic patterns of reactivated TB are more frequently encountered in HIV-infected patients in whom cell-mediated immunity is not deranged.

Although Table 3 does not show the relationship to be significant, tuberculous effusions occur more commonly among those with higher cell counts [56, 81]. The presence of pulmonary infiltrates correlates significantly to higher CD4+ counts (Table 3).

## Diagnosis of tuberculosis

A variety of diagnostic tools exist to detect tuberculosis although a high clinical index of suspicion remains the *sine qua non* of any attempt at making an early diagnosis of TB. Clinical suspicion must, however, correlate with laboratory findings to warrant the commencement of anti-tuberculous regimen.

### *Sputum smear*

Sputum smear and microscopy is the easiest, cheapest and most widely available diagnostic tool for *Mycobacterium tuberculosis*. On the other hand, it has the disadvantage of having a low sensitivity attributable to the fact that the procedure requires between 10,000 to 100,000 organisms/μL of specimen to be sensitive. Another pitfall associated with the smear is its lack of discriminant ability with respect to the various mycobacteria since all of them stain the same. Nevertheless, it remains among the most useful steps in the evaluation of any suspected case of tuberculosis. The sensitivity of the procedure ranges from 45.7% to 61%, while it is reported to be more than 99% specific with a positive predictive value of 91.5% to 98.5% [82–85].

There appears to be a correlation between clinical presentation and smear yield in patients with tuberculosis. Smear positivity correlates directly with cavitary disease as observed by Greenbaum and colleagues [85] who reported positive smears in 52% of patients with cavitary disease but in only 32% with local infiltrates. Similarly, Klein and associates [86] found the yield of sputum microscopy in patients coinfected with HIV and tuberculosis to be 45%, as against 81% in a non-AIDS comparison group. This could be explained by the fact that patients with AIDS tend to have a high incidence of non-cavitary disease [69].

### *Sputum culture*

The yield of sputum cultures for detecting *M. tuberculosis* is higher than that of microscopic examination. Among 435 patients with pulmonary TB, Levy and colleagues [82] found sensitivity and specificity for sputum cultures of 81.5% and 98.4% respectively. The presence of at least 500 organisms is required to have a positive culture. Even though cultures have the advantage of being much more sensitive than smears, they take a longer time to grow, up to 8 weeks for solid media and 1 to 3 weeks for liquid media. In addition, culture is costly and technically not as easy to perform. The delay in the availability of results could lead to patients not being started on a presumptive basis. Similar to smear, culture yield seems to be affected by the clinical presentation of the patient; Greenbaum and associates [85] found that 96% of patients with cavitary disease had positive cultures, compared with only 70% with focal infiltrates.

In order to reduce the time it takes for culture results to be available, enhanced broth-based culture detection and identification systems have been developed, the most widely used of which is the BACTEC system [87]. This technique employs a $^{14}C$-labeled metabolic substrate, palmitic acid, which in the presence of viable mycobacteria is metabolized to $^{14}CO_2$. The amount of radioactive $CO_2$ released in the culture vial can be quantitated and used to detect mycobacterial growth well before conventional cultures would be positive. The BACTEC system is quicker than the agar medium, and time to identification of a positive culture can be shortened to as little as 2 weeks. Distinguishing between *M. tuberculosis* from other mycobacterial species is accomplished presumptively by selective growth in the presence of *par-nitro-α-acetyl-amino-β-hydroxypropiophen-one*. Definitive rapid identification of mycobacteria is available with species-specific nucleic acid probes (e.g. GenProbe) or by analysis of cell wall lipids by gas liquid chromatography.

Response to anti-tuberculosis therapy using sputum culture conversion as a yardstick has been found to be significantly less favorable in HIV-infected than in HIV-uninfected tuberculosis patients [88]. Tuberculosis patients coinfected with the human immunodeficiency virus (HIV) took a significantly longer period of follow up for documented conversion as compared to HIV-uninfected cases, thereby highlighting the importance of longer and more careful control of tuberculosis treatment in HIV-positive patients.

### *Bronchoscopy*

It is not entirely clear as to whether this invasive procedure has a definitive contributory role in establishing the diagnosis of tuberculosis. In a retrospective analysis of 114 patients (67 HIV-positive and 47 HIV-negative), an immediate diagnosis of tuberculosis was obtained in 25 of 66 patients (38%) who had had a negative sputum acid-fast result prior to bronchoscopy. There were no significant differences in the yield based on HIV serology status [89]. However, most of the incremental increase in rapid diagnosis from bronchoscopy was obtained by demonstrating granulomata on transbronchial biopsy. The pitfall of bronchoscopy includes the fact that it is an invasive procedure carrying with it the risks of bleeding and pneumothorax. It is also a costly method besides being a potential source of nosocomial transmission of tuberculosis to bronchoscopists and technicians conducting the procedure.

### *Serodiagnosis*

One obvious advantage of this laboratory procedure is its non-invasive nature, even though studies have found that it does not add to the diagnostic yield in cases where sputum smears are available [90]. The test is based on the detection of specific antigens from *M. tuberculosis* from the patient's blood. Examples of this test are enzyme-linked immunosorbent assay (ELISA) and hemagglutination assay for glycolipid antigens. Currently, serodiagnosis is not widely utilized clinically in the United States.

### *Molecular-based procedures*

The polymerase chain reaction (PCR) has recently gained a lot of attention, and promises to be a powerful and reliable diagnostic tool in the future. Diagnostic PCR is a technique of DNA amplification that uses specific DNA sequences and serve as markers for the presence of micro-organisms and is, in theory, capable of detecting even a single strand of nucleic acid from TB, to amplify it and within a few hours to identify the presence of TB bacilli in a biologic specimen such as sputum, lavage fluid, cerebrospinal fluid, pleural fluid, or blood [91]. The PCR procedure involves, as first step, heating the specimen that contains the organism of interest to denature double-stranded DNA. Then, specific synthetic oligonucleotides, or "primers" (short, single-stranded pieces of DNA) bind to DNA sequences of a target organism that are unique to it or its species, and a heat-stable DNA polymerase then extends the primers to create a complete and complementary strand of DNA. The process is typically repeated sequentially leading to the production of millions of copies of the target DNA sequence. These amplified sequences can then be easily detected by gel electrophoresis. If the target organism is not present in the sample being examined, the primers have nothing to bind to, and no amplification occurs.

The diagnosis of tuberculosis using this nucleic acid amplification (NAA) method became feasible only when specific mycobacterial genetic sequences were identified. The genetic marker most commonly used by investigators is that of the mycobacterial insertion element IS6110, a DNA sequence (of uncertain functional significance) that is present in *M. tuberculosis* as well as in the other members of the *M. tuberculosis* complex (*Mycobacterium africanum*, *Mycobacterium microti*, and *Mycobacterium bovis*) [92]. Studies using this technique have shown sensitivities of 60 to 95% [93] and specificity of up to 99% [94]. The test is most specific when used on smear-positive cases, and for this reason, the Food and Drug Administration approved the test for use on smear-positive, untreated cases. The most promising application of PCR in the diagnosis of tuberculosis may be in pleural or extrapulmonary cases, particularly tuberculous meningitis, in which a positive PCR result would be overwhelming evidence of active infection or dissemination [95].

Disadvantages of the procedure include cost and technical sophistication thereby limiting its utility in developing countries. Also, positive results will require the additional procedure of culture to grow the organism in order to test their sensitivity to anti-tuberculous drugs. Nevertheless, the merits of the technique outweigh its pitfalls.

## Treatment of tuberculosis

Studies have shown that the standard six-month regimen for the treatment of tuberculosis results in prompt sterilization of sputum [96, 97] although other investigators have reported higher rates of relapses in HIV-infected patients who received 6 as compared with 9 or 12 months of anti-tuberculosis chemotherapy [98, 99]. The increased incidence of relapses among HIV-co-infected patients, which is an indication of treatment failure, could be as a result of a number of factors. Even though anti-retroviral combination regimens have dramatically improved the prognosis for HIV-infected patients [100, 101], they have also complicated the management of tuberculosis. Rifampicin induces the activity of cytochrome P-450 CYP3A, which lowers the concentration of HIV-protease inhibitors and non-nucleoside reverse-transcriptase inhibitors to subtherapeutic levels. Low trough plasma levels of these anti-retroviral drugs are associated with incomplete viral suppression and emergence of drug resistance [102, 103]. Furthermore, rifampicin monoresistant tuberculosis is more frequent in HIV-infected patients than in HIV-seronegative patients [104], and most cases arise independently from mutations in drug-susceptible strains, not from extensive transmission of a few rifampicin-monoresistant strains [104, 105]. Secondary rifampicin monoresistant tuberculosis is independently related to therapy nonadherence, severe immunosuppression, positive acid-fast sputum smear, concomitant anti-fungal therapy and diarrhea [104, 106]. The mechanism of rifampicin monoresistance is still poorly understood.

Rifabutin is a weaker inducer of cytochrome P-450 CYP3A and is recommended as an alternative to rifampicin for the treatment and prophylaxis of tuberculosis in HIV [107]. However, its combined administration with the protease inhibitor indinavir results in not only a significant decrease in indinavir concentrations with subsequent risk of treatment failure, but also leads to a significant increase in rifabutin concentrations with increased risk of toxicity [108, 109].

Another important factor that might compromise treatment of tuberculosis in HIV-positive individuals is drug malabsorption. Two studies among HIV-infected persons with tuberculosis have yielded conflicting results. In one study, the plasma levels of rifampicin and ethambutol were found to be lower in HIV-infected patients with tuberculosis than in historical control patients with tuberculosis [110], while another study reported no differences between HIV-positive and HIV-negative TB patients, in terms of the peak level or total absorption of isoniazid, rifampicin or pyrazinamide [111]. Therefore, the impact of anti-tuberculous drug malabsorption on treatment response among HIV-infected patients remains speculative.

In immunedepleted patients TB may show a paradoxical worsening upon institution of HAART and TB treatment, a phenomenon also known as "immune recovery syndrome" [112]. The pathogenesis of this phenomenon is believed to be related to the development of improved *M. tuberculosis*-specific immune responses during the course of anti-TB treatment. Paradoxical worsening is an important consideration in the clinical management of TB because such cases tend to be associated with a higher rate

of TB relapse than those not complicated by paradoxical worsening, and may require longer duration of therapy than the standard 6-month rifamycin-based treatment [113, 114].

Studies comparing treatment response in HIV-infected and HIV-uninfected tuberculosis patients have been confined to clinical or hospital settings. Such investigations, even though very well conducted, may be limited in terms of generalizability. Adopting a population-based approach could circumvent such a limitation. Another advantage of a population-based study in this regard, is that it allows gauging the effectiveness rather than the efficacy of anti-tuberculosis therapy in HIV-positive relative to HIV-negative tuberculosis patients.

## Mortality due to HIV-TB coinfection

In persons co-infected with the human immunodeficiency virus and tuberculosis, there is increased mortality despite adequate therapy [115, 116]. Studies conducted in both developed and developing countries (Table 4) have demonstrated overwhelming evidence that the occurrence of tuberculosis in an HIV-setting is significantly associated with poor prognosis for survival (Table 4). Co-infected patients have a 4- to 8-fold higher likelihood of death as compared to HIV-negative TB patients [42, 116], and the magnitude of excess mortality attributable to HIV infection, or the attributable fraction, is about 87% in the United States [42].

Predictors of survival in HIV patients with tuberculosis include multi-drug resistance, the status of the immune system as measured by CD4+ count, the stage of HIV infection at the time of diagnosis, appearance of chest X-ray at presentation, cutaneous anergic response, site of tuberculosis, delayed or lack of treatment and old age.

Multi-drug resistance has been found to be an independent predictor of survival in tuberculosis patients in general (adjusted relative risk = 5.8; 95% CI = 2.3–14.6, compared with pansensitive cases) but its impact on co-infected persons with HIV is more profound than in HIV-negative TB patients (cumulative mortality of 92% versus 63%) [117]. The interaction between the immune system of HIV patients and the progression of tuberculosis leading to early mortality is vividly depicted by the excess mortality among HIV-infected individuals with significant CD4+ lymphocytopenia. Among HIV-TB co-infected pa-

**Table 4.** Pooled results of studies estimating the relationship between HIV and mortality in patients with tuberculosis

| Investigators | Mortality rate | | Time to censorship | Site and type of study |
|---|---|---|---|---|
| | HIV+ | HIV– | | |
| Perriëns et al. (1991) [118]<br>Sample size | 31%<br>150 | 4%<br>501 | 1 year | Zaire. Longitudinal study.<br>Community-based. |
| Stoneburner et al. (1992) [42]<br>Sample size | 83%<br>31 | 11%<br>27 | 2.5 years | US. Longitudinal study.<br>Hospital-based. |
| Nunn et al. (1992) [115]<br>Sample size | 21%<br>107 | 6%<br>174 | 6 months | Kenya. Prospective study.<br>Hospital-based. |
| Ackah et al. (1995) [111]<br>Sample size | 6%<br>180 | 0.4%<br>280 | 6 months | Ivory Coast. Prospective study.<br>Hospital-based. |
| Richter et al. (1995) [119]<br>Sample size | 22%<br>102 | 2%<br>55 | 1 year | Tanzania. Prospective study.<br>Clinic-based. |
| Perriëns et al. (1995) [98]<br>Sample size | 31%<br>260 | 2%<br>186 | 2 years | Zaire. Prospective study.<br>Population-based. |
| Whalen et al. (1995) [120]<br>Sample size | 35%<br>106 | –<br>– | 1 year | US. Retrospective cohort study.<br>Hospital-based multi-center. |
| Pablos-Mendez et al. (1996) [117]<br>Sample size | 40%<br>114 | 15%<br>115 | 1 year | US. Observational study.<br>Population-based. |
| Whalen et al. (1996) [116]<br>Sample size | 32%<br>191 | –<br>– | 1 year | Uganda. Prospective study.<br>Community-based. |
| Whalen et al. (1995) [120]<br>Sample size | 36%<br>112 | –<br>– | 1 year | US. Retrospective cohort study.<br>Hospital-based multi-center. |
| Connolly et al. (1999) [122]<br>Sample size | 41%<br>214 | 19%<br>189 | 2 years | South Africa. Prospective cohort study. Hospital-based. |
| Murray et al. (1999) [123]<br>Sample size | 14%<br>190 | 0.5%<br>186 | 6 months | South Africa. Prospective cohort study. Population of gold-miners. |
| Whalen et al. (2000) [124]<br>Sample size | 28%<br>230 | –<br>– | 19 months | Uganda. Prospective cohort study.<br>Community-based. |
| Pooled Mortality<br>95% CI | 29%<br>(26.8–30.8%) | 6%<br>(4.0–7.1%) | | |

tients with CD4+ count below 200 cells/μL, mortality at 6-month to 1-year is two to three times higher than the death rate among those with higher CD4+ count [111, 125]. It is known that infection of monocytes with *M. tuberculosis* enhances replication of HIV [126], perhaps because the presence of *M. tuberculosis* antigens induces monocytes to secrete tumor necrosis factor-α [127, 128], which in turn stimulates proliferation of HIV [129]. This creates and establishes a vicious cycle that culminates in the early demise of patients co-infected with tuberculosis and HIV.

Superimposed opportunistic infections jeopardize survival of patients with TB-HIV comorbidity even after correcting for the confounding effect of lowered immune status [130]. Autopsy examination has shown that in HIV-positive patients with TB as well as opportunistic infections, early mortality is more likely to be due to tuberculosis, whereas, late deaths are more commonly as a result of opportunistic infections such as cryptococcal pneumonia [123]. This complements the finding from other investigators that active tuberculosis exerts its greatest effect on survival in the early stages of HIV infection, when there is a reserve capacity of the host immune system [124].

It has also been found that less extensive disease on chest X-ray (such as cavitation) among HIV-TB co-infected patients is a marker for poor survival outcome in these patients [109]. Presentation with an atypical pattern on chest X-ray is associated with a shorter survival and a two-fold increase in the risk of death [110]. The direct relationship between mortality and less extensive radiologic changes may be explained by the fact that much of the radiologic shadowing seen in pulmonary tuberculosis represents the effects of intact cell-mediated immunity: inflammation, fibrosis and cavity formation [115].

Response to tuberculin skin test is a function of intact CD+ lymphocytes, macrophages and cytokines [131]. Poor survival is associated with cutaneous anergy to purified protein derivative (PPD) in HIV-infected persons with tuberculosis [116]. Even in HIV-infected patients without tuberculosis, cutaneous anergy to tuberculin PPD is not only a marker of cellular immune function but also of survival, independent of CD4+ cell count [132].

Increased mortality correlates with the site of tuberculosis disease [133]. The mortality rate is higher in HIV patients with extrapulmonary tuberculosis foci than in those with only pulmonary involvement [133]. Some investigators have also suggested that the site of extrapulmonary disease is an important prognostic factor, particularly, in tuberculosis affecting the meninges, blood or bone marrow [121]. Tuberculous meningitis accounts for about 10% of tuberculosis disease in patients that are HIV-positive [134]. Mortality rate in meningitic TB in HIV persons is about 44%, and the risk of death from tuberculous meningitis is four times higher than in patients with pulmonary involvement alone [121]. Older age and lack of treatment have also been noted as poor predictors of survival in HIV-TB co-morbidity setting [117].

## Summary

The mortality associated with HIV-TB coinfection can be substantially reduced by strengthening current control strategies through expansion of DOTS, increased active case finding (evaluate and treat contacts) and identifying latently infected persons as well as providing preventive therapy. Increasing HIV counseling and testing and creating the infrastructure to deliver HAART are also important in this regard.

Despite major gains that have been made in the past two decades, important questions still remain. Additional research is needed in the areas of mechanisms to ensure adequate drug supply, proper surveillance and program coordination, and in the development of more potent, less toxic, faster acting anti-TB drugs and regimens. Further research in the design of faster, cheaper point-of-use tests for diagnosis of TB in HIV+ people and for detection of drug resistant TB strains as well as development of better TB vaccines will contribute to slowing down this dual epidemic.

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