Tuberculosis

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Tuberculosis results in an estimated 1·7 million deaths each year and the worldwide number of new cases (more than 9 million) is higher than at any other time in history. 22 low-income and middle-income countries account for more than 80% of the active cases in the world. Due to the devastating effect of HIV on susceptibility to tuberculosis, sub-Saharan Africa has been disproportionately affected and accounts for four of every five cases of HIV-associated tuberculosis. In many regions highly endemic for tuberculosis, diagnosis continues to rely on century-old sputum microscopy; there is no vaccine with adequate effectiveness and tuberculosis treatment regimens are protracted and have a risk of toxic effects. Increasing rates of drug-resistant tuberculosis in eastern Europe, Asia, and sub-Saharan Africa now threaten to undermine the gains made by worldwide tuberculosis control programmes. Moreover, our fundamental understanding of the pathogenesis of this disease is inadequate. However, increased investment has allowed basic science and translational and applied research to produce new data, leading to promising progress in the development of improved tuberculosis diagnostics, biomarkers of disease activity, drugs, and vaccines. The growing scientific momentum must be accompanied by much greater investment and political commitment to meet this huge persisting challenge to public health. Our Seminar presents current perspectives on the scale of the epidemic, the pathogen and the host response, present and emerging methods for disease control (including diagnostics, drugs, biomarkers, and vaccines), and the ongoing challenge of tuberculosis control in adults in the 21st century.

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Introduction

Tuberculosis has plagued humankind worldwide for thousands of years. John Bunyan (Nov 28, 1628–Aug 31, 1688), an English Christian writer and preacher, described tuberculosis as "The Captain among these men of death" at a time when tuberculosis case rates in London had reached 1000 per 100 000 population per year.[1] Tuberculosis continued to cause many deaths in London during the 19th century and accounted for up to 25% of deaths in Europe. The death toll from tuberculosis began to fall as living standards (housing, nutrition, and income) improved early in the 20th century, well before the advent of antituberculosis drugs. Despite the first antituberculosis drugs being discovered more than 60 years ago, tuberculosis today still kills an estimated 1·7 million people each year.[2] Progress in the scaling up of tuberculosis diagnostic, treatment, and control efforts worldwide over the past decade has been associated with improvements in tuberculosis control in many parts of the world, but progress has been substantially undermined by the HIV-1 epidemic, the growing challenge of drug resistance, and other increasingly important epidemiological factors that continue to fuel the tuberculosis epidemic.[3] Greater investment in new technologies, basic science, and translational and applied research has led to progress in the development of improved tuberculosis diagnostics, drugs, treatment regimens, biomarkers of disease activity, and vaccines; new perspectives in the pathogenesis of tuberculosis are also emerging. Our Seminar focuses on tuberculosis in adults and presents current perspectives on the scale of the epidemic, the pathogen and host response, current and emerging methods for disease control (including diagnostics, drugs, biomarkers, and vaccines), and the ongoing challenge of tuberculosis control in the 21st century.

Epidemiology

The estimated total number of incident cases of tuberculosis worldwide rose to 9·4 million in 2009—more than at any other time in history.[4] The worldwide tuberculosis incidence rates are estimated to have peaked in 2004 and to have decreased at a rate of less than 1% per year since that time. However, the overall worldwide burden continues to rise as a result of the rapid growth of the world population. Most cases are in Asia and Africa, with smaller proportions of cases in the eastern Mediterranean region, European region, and the Americas (figure 1).[4] 22 countries account for 80% of the worldwide burden and the five countries that rank first to fifth in the world in terms of total numbers of incident cases in 2009, were India, China, South Africa, Nigeria, and Indonesia.

About 12% (1·1 million cases) of the worldwide tuberculosis caseload was HIV-associated and most of

Search strategy and selection criteria

Our Seminar is focused on tuberculosis in adults. Readers are referred to recent reviews on tuberculosis in children. Our search strategy included a 7-year review of PubMed (2004–11), the Cochrane library (2004–10), WHO and WHO-STOP TB publications (2000–10), and Embase (2004–10), and three recent comprehensive tuberculosis textbooks (Tuberculosis: a comprehensive clinical reference [Philadelphia, PA: Saunders, 2009]; Tuberculosis: the essentials, 4th edn [London: Informa, 2009]; Handbook of tuberculosis—vols 1, 2, and 3 [Hoboken, NJ: John Wiley and Sons Inc, 2008]). The search terms used were "tuberculosis", "*Mycobacterium tuberculosis*", "tuberculosis" and "HIV", "immunity", "pathogenesis", "clinical features", "diagnosis", "diagnostic tests", "biomarkers", "imaging", "radiology", "treatment", "prevention", "latent infection", "vaccines", "control", "drug-resistant", "extensively drug-resistant", "pregnancy", "vulnerable groups", "prevention", "research priorities", "packages of care". We also included commonly referenced older published work on tuberculosis, and cite so-called state of the art tuberculosis review articles, to provide more details and references than cited in our Seminar.

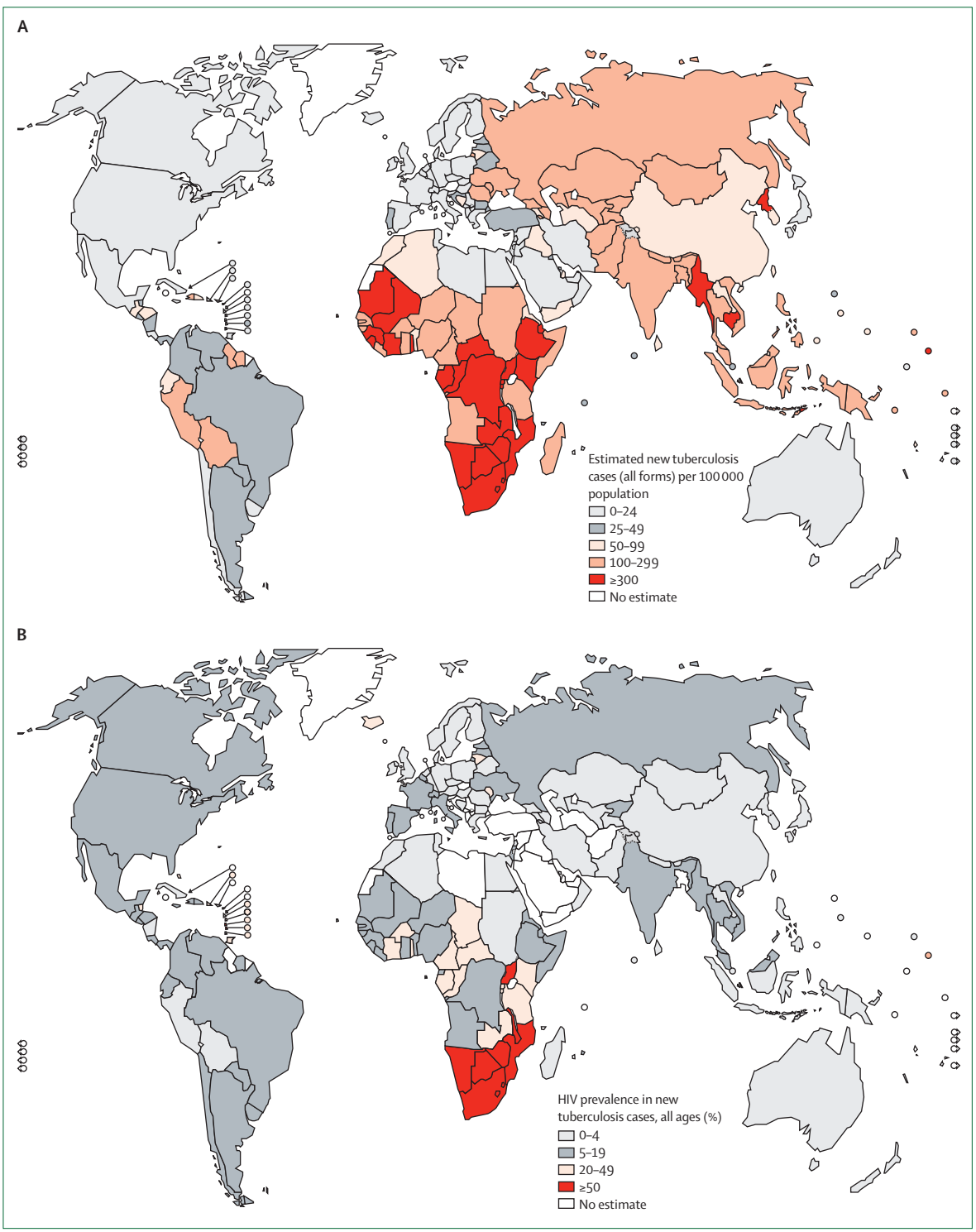

Figure 1: **Tuberculosis incidence and prevalence rates in 2009**
Estimated tuberculosis incidence rates (A). Estimated HIV prevalence in new tuberculosis cases (B). Reproduced from Global tuberculosis control.[4]

these cases were in sub-Saharan Africa (about four of every five cases) and southeast Asia (figure 1).[4] HIV has fuelled a three to five times increase in tuberculosis incidence rates in many high HIV prevalence countries in sub-Saharan Africa (figure 2), especially in the south and east of the continent.[2,5] In the worst affected countries of South Africa and Swaziland, about 1% of the population develops tuberculosis each year, much of it due to HIV.

The collapse of the Soviet Union and the multidrug-resistant (MDR) tuberculosis epidemics in marginalised populations such as prisoners and drug abusers have been key factors underlying the increases in tuberculosis incidence rates noted in eastern Europe (figure 2).

Tuberculosis remains a disease of poverty that is inextricably associated with overcrowding and undernutrition.[6,7] The table shows a range of other risk factors for tuberculosis. Infection with HIV is the most potent of these risk factors, with the risk of people infected with HIV developing tuberculosis being more than 20-times greater than that of people not infected with HIV.[8] Other risk factors include heavy alcohol consumption and smoking; the latter roughly doubles risk of tuberculosis[27] and might account for up to half of all deaths in men with tuberculosis in India.[14] Diabetes is associated with an about three-times increase in tuberculosis risk (table) and accounted for about 20% of smear-positive tuberculosis cases in India in 2000.[28] Immunosuppressive drugs such as corticosteroids have long been associated with the risk of tuberculosis, but tuberculosis associated with tumour necrosis factor (TNF) antagonists for the treatment of rheumatological disorders is now an increasing problem in industrialised countries.[26] The evidence for a human genetic contribution to susceptibility to tuberculosis is now growing (table).[24] Genetic variants known to affect susceptibility include the natural resistance-associated macrophage protein (NRAMP), the vitamin D receptor (VDR), and the nitric oxide synthase (NOS2A) and interferon-γ pathways. Although the effect size for most of these associations is only moderate, the cumulative effect of these polymorphisms to the burden of tuberculosis in different populations could be substantial but remains to be defined.[3]

Over the past two decades there has been the worldwide emergence of MDR tuberculosis, then extensively resistant (XDR) tuberculosis, and, most recently, strains that are resistant to all antituberculosis drugs.[29–32] MDR tuberculosis is caused by *Mycobacterium tuberculosis* that is resistant to at least isoniazid and rifampicin, and XDR tuberculosis is caused by MDR tuberculosis strains that are also resistant to any fluoroquinolone and one of three injectable aminoglycosides (capreomycin, kanamycin, and amikacin). It is estimated that there were about 0·5 million incident cases of MDR tuberculosis and 50 000 cases of XDR tuberculosis in 2007.[2] Of the cases of MDR tuberculosis, about 0·3 million were new cases (primary drug resistance) and 0·2 million were patients previously treated for tuberculosis (acquired drug resistance).[2]

The countries ranked first to fifth in terms of total numbers of drug-resistant cases were India, China, the Russian Federation, South Africa, and Bangladesh. MDR tuberculosis accounts for 5·7% of new cases and 25·6% of previously treated cases in China, and together China and India account for about 50% of the total worldwide burden of MDR tuberculosis.[32] In parts of the Russian Federation however, MDR tuberculosis accounts for as much as a quarter of new cases of tuberculosis[32] and is a crucial problem in prisons in eastern Europe[33] and sub-Saharan Africa.[34] By March, 2010, 58 countries had reported at least one case of XDR tuberculosis to WHO.[32] The intersection of the drug-resistant tuberculosis and HIV epidemics further threatens to undermine tuberculosis control, creating a so-called perfect storm in regions where the prevalence of these is high, such as in KwaZulu Natal province, South Africa.[35]

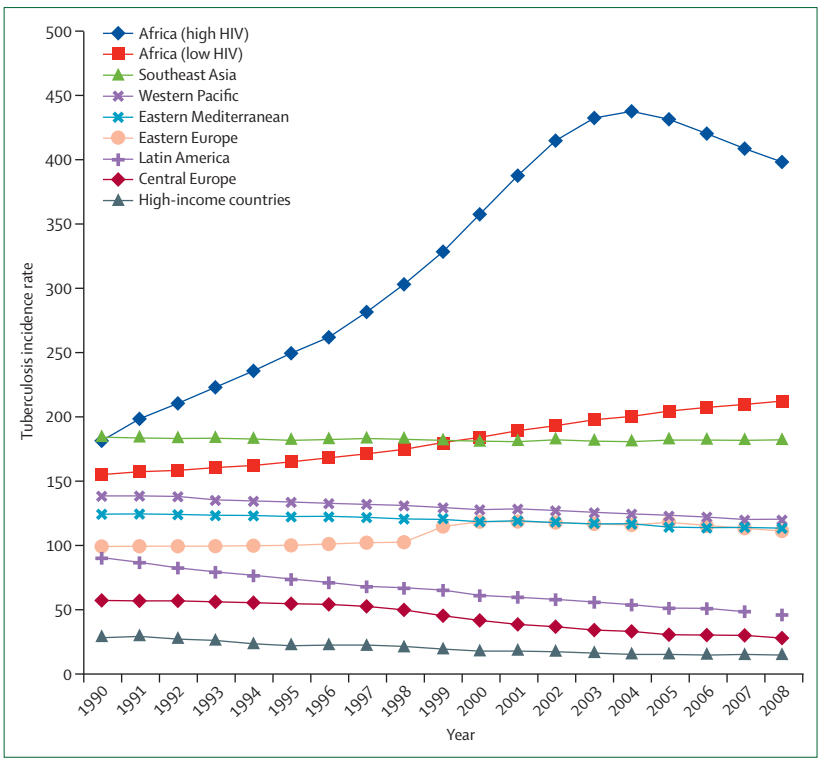

Figure 2: **Trends in estimated tuberculosis incidence rates in nine subregions, 1990–2008**
Data from Global tuberculosis control—a short update to the 2009 report.[2]

Microbiology of *Mycobacterium tuberculosis*

M tuberculosis was first identified by the German scientist Robert Koch (figure 3), who announced the discovery on March 24, 1882. The *M tuberculosis* complex of organisms, which can cause human disease, consists of *M tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*, and *Mycobacterium canetti*. *M bovis* was responsible for about 6% of all human tuberculosis deaths in Europe before the introduction of milk pasteurisation; subsequent attenuation of a laboratory strain of *M bovis* led to the development of the BCG vaccine in 1921.

M tuberculosis is an obligate intracellular pathogen that can infect several animal species, although human beings are the principal hosts.[36] It is an aerobic, acid-fast, non-motile, non-encapsulated, non-spore forming bacillus. It grows most successfully in tissues with high oxygen content, such as the lungs. Compared with the cell walls of other bacteria, the lipid-rich cell wall is relatively

	Effect
HIV[5,8]	Greatly increased susceptibility to infection, primary progressive disease, reactivation, and recurrence; disease incidence rate ratio of between 20 and 37 for people infected with HIV depending on country HIV prevalence
Diabetes[9,10]	About three-times increased risk of tuberculosis (especially in insulin-dependent disease); higher mortality
Undernutrition and vitamin deficiencies[11,12]	Undernutrition, low body-mass index, and vitamin D deficiency are each associated with increased risk of tuberculosis disease
Overcrowded living conditions[13]	Increased exposure to infectious cases
Smoking[14-16]	About two-times increased risk of infection, progression to tuberculosis disease and death
Indoor air pollution[15]	About two-times increased risk of disease (weak evidence)
Silicosis[17]	About three-times greater risk in South African gold miners with silicosis
Alcohol[18]	About three-times increased risk of disease associated with consumption >40 g per day
Sex[19]	The ratio of incident tuberculosis disease in men:women is about 2:1 in adults but not children
Age[20]	Major effect on risks of acquisition, disease progression, form of disease, and mortality risk
End-stage renal failure[21]	More than ten-times increased risk
Malignancy[22,23]	Both solid organ and haematological malignancies associated with increased risk
Genetic susceptibility[24]	There is a growing list of genes associated with risk of tuberculosis, including genes for natural resistance-associated macrophage protein 1, interferon γ, nitric oxide synthase 2A, mannan binding lectin, vitamin D receptor, and some Toll-like receptors
TNF antagonist therapy[25,26]	Risk of tuberculosis disease increased about one and a half times in rheumatology patients in North America; risk greater with TNF antibodies than with soluble TNF receptor
Corticosteroid therapy[25]	Risk of tuberculosis disease increased about two times in rheumatology patients in North America

TNF=tumour necrosis factor.

Table: **Risk factors associated with tuberculosis**

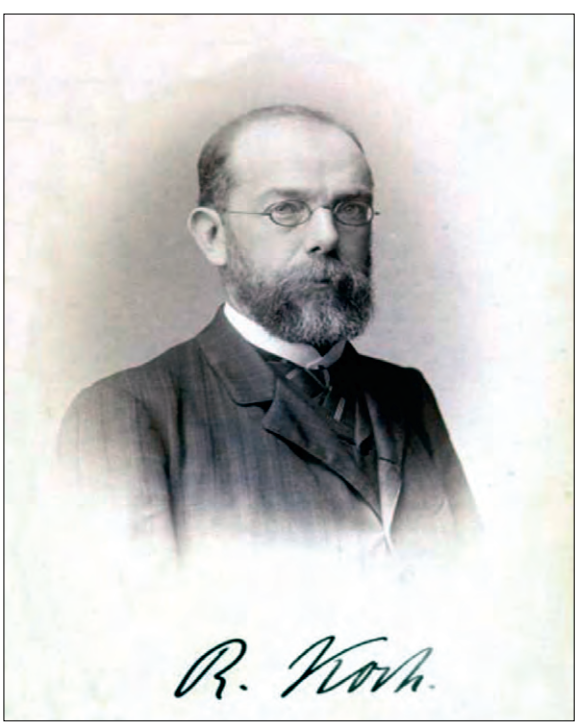

Figure 3: **Robert Koch (Dec 11, 1843–May 27, 1910)**
Photograph provided by Stefan Kauffman, Max Planck Institute, Berlin.

impermeable to basic dyes unless combined with phenol. Thus, *M tuberculosis* is neither gram positive nor gram negative but is instead described as acid-fast, since once stained it resists decolourisation with acidified organic solvents (figure 4). Since other bacteria, such as non-tuberculous mycobacteria and *Nocardia* spp, also contain mycolic acids, they are also acid-fast and cannot be distinguished from *M tuberculosis* on microscopic sputum smear examination. *M tuberculosis* divides every 15–20 h, which is extremely slow compared with other bacteria (*Escherichia coli* divides every 20 minutes). This slow replication rate and ability to persist in a latent state result in the need for long durations of both drug therapy of tuberculosis and for preventive therapy in people with *M tuberculosis* infection.

Although many questions remain unanswered about the origins of *M tuberculosis*, advances in mycobacterial genomics are now providing evidence that the amount of sequence variation in the *M tuberculosis* genome might have been underestimated and that some genetic diversity does have important phenotypic consequences.[37,38] Although there are many *M tuberculosis* strains, studies of the phylogeny and biogeography of *M tuberculosis* have revealed six main strain lineages that are associated with particular geographical regions.[38] Much research is focused on elucidating possible differences between strains with regard to transmission and pathogenesis. It is speculated that the Beijing family of strains originated in Asia and the strain *W* and strain *W*-like families are responsible for many cases of drug resistance. This family of strains is distributed worldwide and is able to spread in large clonal clusters. However, it has not been defined if this family has a genetic advantage that enables it to spread, cause disease, and develop drug resistance.[39] The sequencing of the *M tuberculosis* genome was a major step forward towards increasing our overall understanding of the bacterium[40] and this has subsequently led to identification of specific antigens for the development of new diagnostics tests, vaccines, and biomarkers for tuberculosis.

Host–pathogen interactions

The yearly probability of developing active clinical tuberculosis after inhalation of an *M tuberculosis* aerosol from an infectious patient with active tuberculosis is very small, with an estimated lifetime risk of about 10%.[27] The risk of transmission is highest within the first few years after infection, but decreases substantially thereafter. Most immunocompetent individuals (over 90% of those infected) either eliminate *M tuberculosis* or contain it in a latent state. So-called latent tuberculosis is a clinical disorder in individuals infected with *M tuberculosis* in whom the host immune system retains sufficient control over replication of the bacterium such that the individual remains free of tissue damage and symptoms. The presence of viable *M tuberculosis* bacilli in such individuals has been shown by culturing the organism from tissues obtained from healthy individuals who died from traffic accidents but who had no macroscopic or histological evidence of tuberculosis.[41]

An estimated 2 billion people worldwide have latent *M tuberculosis* infection.[42] Despite the great importance of this enormous reservoir of potential disease, the interactions of *M tuberculosis* with the human host that mediate clinical latency are largely unknown. With advances in technology, our understanding of pathogenesis and protective immune responses to infection with *M tuberculosis* is constantly growing.[43] *M tuberculosis* has evolved elaborate survival mechanisms in human beings that allow it to remain in a clinically latent state, although the mechanisms of persistence remain incompletely defined. The high rates of clinical tuberculosis in people infected with HIV and in those with various inherited defects of the interferon-γ signalling pathway indirectly suggest a key role for the adaptive immune responses after antigen recognition by specific T cells.[44,45]

However, the first interaction between *M tuberculosis* and the host is with the innate immune system and seems to be mediated by pattern recognition receptors. Recognition by macrophages and dendritic cells of the biochemical products of *M tuberculosis* such as mannosylated lipoarabinomannan,[46] trehalose dimycolate,[47] and N-glycolyl muramyl dipeptide[48] trigger innate responses and might be important in establishing ensuing host–pathogen interactions. However, the final effector pathway remains unclear. In addition to macrophage activation, a potential role for neutrophils in the innate immune response is also emerging. Studies suggest that neutrophils are not simply scavenging phagocytic cells but are infected with *M tuberculosis* in the sputum and within cavities of patients with active tuberculosis.[49] Several antimicrobial peptides, such as cathelicidin LL37, produced by neutrophils, have activity against *M tuberculosis*.[50] Uptake of *M tuberculosis*-induced apoptotic neutrophils by macrophages also triggers macrophage activation, which provides a subsequent link between the innate and acquired immune response.[51] Furthermore, there is growing interest in the link between susceptibility to tuberculosis and vitamin D deficiency; this has pleiotropic effects on the immune system,[52] including macrophage activation and induction of the antimycobacterial peptide LL37.[53] Vitamin D supplementation might enhance antimycobacterial immune function in vitamin D deficient populations. A randomised clinical trial[54] showed that vitamin D supplementation was associated with more rapid sputum-culture conversion in a subset of individuals with specific vitamin D receptor polymorphisms and further clinical trials of vitamin D supplementation in the treatment and prevention of tuberculosis are warranted.

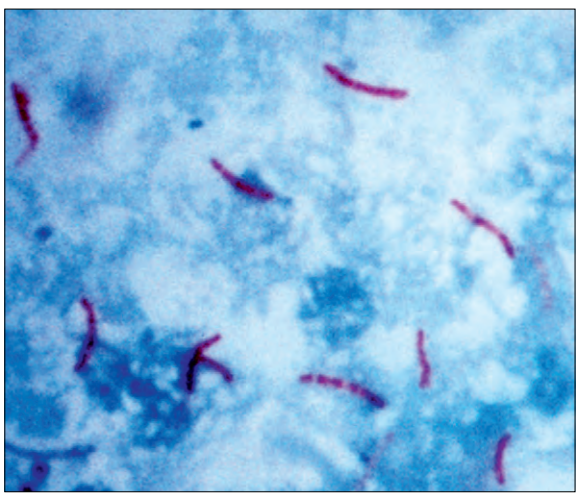

Figure 4: **High-power micrograph of acid-fast bacilli in the sputum of a patient with tuberculosis, shown by Ziehl-Neelsen staining (×1000)**

Conventional dogma, supported by an extensive body of research, has focused on cell-mediated immunity, with T lymphocytes and macrophages regarded as having the dominant role in protective immunity to *M tuberculosis*.[43,55] In immunocompetent individuals, this culminates in the formation of granulomas, which are highly effective in containing, but not eliminating, the infection.[55–57] These granulomas have a dominant role in the pathogenesis of *M tuberculosis* and other intracellular pathogens (figure 5) and are defined as focal, compact collections of inflammatory cells in which mononuclear cells dominate and are usually formed as a result of an undegradable product, microorganisms, or hypersensitivity reaction.[56] Recent molecular studies suggest that mycobacteria might promote cellular recruitment to the granuloma, which further suggests that granuloma formation is part of a pathogen-directed virulence programme. Therefore, although granuloma formation seems to function as a host defence mechanism, there are also apparent advantages of this response conferred to *M tuberculosis*.[55,58–60]

Within granulomas, *M tuberculosis* might shield itself from immune-based killing mechanisms and escape therapeutic concentrations of antituberculosis drugs, potentially promoting the emergence of drug-resistant

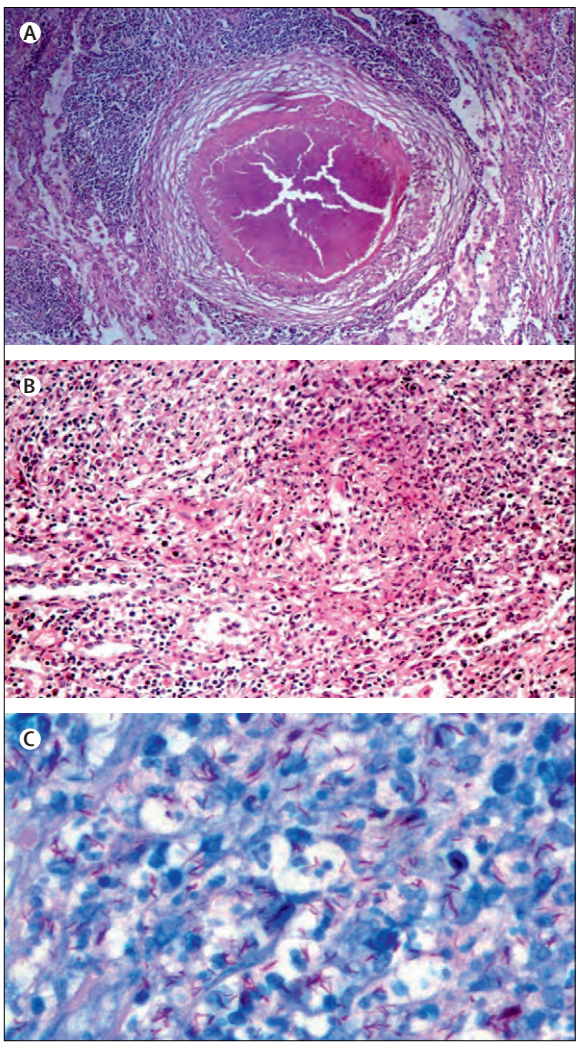

Figure 5: **Micrographs of tissue specimens from patients with tuberculosis**
Low-power micrograph (×100) of a haematoxylin and eosin stained tissue section from an immunocompetent patient with tuberculosis that shows a well formed tuberculous granuloma with a central area of caseous necrosis surrounded by epithelioid macrophages, giant cells, and T lymphocytes, and surrounding outer fibrosis (A). Medium-power (×250) micrograph of tissue from a patient infected with HIV with advanced immunodeficiency that shows mononuclear infiltrate but absence of granuloma formation (B). High-power (×400) tissue section from the same specimen from the patient with advanced immunodeficiency with Ziehl-Neelsen staining showing numerous acid-fast bacilli with little evidence of a cellular immune host response (C). Micrographs provided by Colleen Wright, Stellenbosch University, South Africa.

strains. At a cellular level, the granuloma macrophage might also have two mutually contradictory roles: activated macrophages are capable of killing or controlling the growth of *M tuberculosis*, and yet they also provide the primary growth niche for this intracellular organism.[55,58–60] The fate of infected macrophages has an essential role in protection against *M tuberculosis* by regulating innate and adaptive immunity. Virulent strains of *M tuberculosis* inhibit apoptosis and trigger macrophage necrosis, thereby evading innate immunity and delaying the start of adaptive immune responses.[61]

Granulomas are absent or poorly formed in people with poor immune responses, particularly those infected with HIV (figure 5).[45,62] Through several mechanisms, HIV-1 coinfection leads to functional and numeric depletion of *M tuberculosis*-specific CD4 T lymphocytes and type-1 cytokine production. The resulting dysfunction of the CD4 T lymphocyte–macrophage immune axis impairs the host's ability to orchestrate cell-mediated immune responses and form immunologically competent granulomas.[63] As a result, histological examination of tissue specimens might reveal uncontrolled *M tuberculosis* replication with little evidence of a host cellular response (figure 5). Sites of tuberculosis disease and granulomas themselves provide the ideal microenvironment for the propagation of HIV-1, thereby maximising the adverse consequences of HIV-1 at the crucial interface between *M tuberculosis* and the host.[45]

Our understanding of the host–pathogen dynamics of infection with *M tuberculosis* has fundamentally changed in recent years. The traditional theory that distinguishes latent infection from active disease as distinct binary states is overly simplistic. Granulomas are not fixed inert structures, as previously described; they are very active with constantly changing dynamic structures of metabolically active tissues.[64] It is thought likely that a continuous spectrum of states exists both in the same individual and between different individuals, with varying degrees of immune control and mycobacterial bacillary load,[64–66] and that HIV substantially shifts this spectrum in favour of bacillary replication.[67] In view of this new theory, new biomarkers are needed that can more precisely define these disease states and assess the probability of progression of infection with *M tuberculosis* to active tuberculosis disease.

Biomarkers

High on the tuberculosis research agenda is the discovery of host and pathogen biomarkers of active tuberculosis for diagnosis, monitoring treatment, and assessing outcomes (including cure and relapse). A biomarker is defined as a characteristic that is objectively measured and assessed as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. Biomarkers thus provide information about current health status, future health status, and advance knowledge of pathogenesis. Therefore, they might potentially be used to predict reactivation risk, tuberculosis cure, eradication of latent tuberculosis, and vaccine efficiency, and also provide endpoints for clinical trials. By contrast, a diagnostic test classifies patients at a single timepoint as having active tuberculosis, latent infection with *M tuberculosis*, or neither. A biomarker of set of biomarkers might serve as a diagnostic test.

Biomarkers can be either host or pathogen specific[68,69] and tuberculosis-specific ones are needed to serve as

surrogate endpoints, assisting candidate selection during drug discovery, accelerating dose selection in early clinical studies, shortening the time to licensing of new drugs, and development and assessment of new vaccines for tuberculosis. Although progress has been slow, newer technologies are being used to study large cohorts of patients and have yielded new data.[70–74]

In an attempt to identify new biomarkers, multiplexed assays are now being used to compare gene expression between patients with tuberculosis, healthy people with latent infection, and healthy people with no exposure to *M tuberculosis* (controls).[70,75,76] Several biomarkers, when combined, might be substantially better than any single marker and a small number of studies suggest that specificity and higher predictive values can be achieved by measuring several variables with proteomics, transcriptomics, and metabolomics.[75] Recent transcriptomic studies from South Africa,[76,77] a high tuberculosis endemic area, have identified signatures involving expression profiles of genes in blood cells that could distinguish active tuberculosis, latent infection, cure, and tuberculosis recurrence.

Another study of South African patients with active and latent infection with tuberculosis[70] identified a whole-blood 393-transcript signature for active tuberculosis in intermediate-burden and high-burden settings, correlating with the radiological extent of disease and reverting to that of healthy controls after treatment. The investigators also identified a specific 86-transcript signature that discriminates active tuberculosis from other inflammatory and infectious diseases. Modular and pathway analysis revealed that the tuberculosis signature was dominated by a neutrophil-driven interferon-inducible gene profile. The study provides a broad range of transcriptional biomarkers with potential as diagnostic and prognostic techniques.

Separate studies might be needed to assess biomarkers in people with HIV infection. With accumulation of data from multiparameter assay studies, it is becoming clear that biomarkers, and the associations they generate, are probably specific and restricted to the population under study and might not necessarily be broadly applicable.

Diagnostics

The estimated worldwide detection rate for new sputum smear-positive cases of tuberculosis of 62% in 2008 fell substantially short of the 2005 target detection rate of 70%,[2] and the lack of accurate and rapid diagnostics remains a major obstacle to progress in this regard. Over 90% of the worldwide burden of tuberculosis is in low-income and middle-income countries where the diagnosis of tuberculosis still relies heavily on sputum smear microscopy and chest radiology. These techniques are often unsatisfactory and unavailable at patients' first point of contact with the health system. There is a great need for rapid point-of-care tests that can be readily used at all levels of the health system and in the community. Childhood tuberculosis and sputum smear-negative pulmonary and extrapulmonary tuberculosis in adults remain the greatest diagnostic challenge.

Progress has been made over the past decade to improve existing tuberculosis diagnostics and develop new technologies, some of which have been endorsed by WHO.[69,78] In a meta-analysis,[79] sputum processing with bleach or sodium hydroxide and centrifugation was associated with an average 13% increase in the sensitivity of smear microscopy. Fluorescence microscopy also increases sensitivity by 10%, while retaining similar specificity compared with conventional Ziehl-Neelsen staining.[80] This technology permits much more efficient reading of slides, which is crucial to improving laboratory performance. Although traditional fluorescence microscopes are expensive, much cheaper fluorescence microscopes with light-emitting diodes (LEDs) are equally sensitive and were endorsed by WHO in 2009.[81]

Automated liquid culture systems are now the gold standard for the diagnosis of tuberculosis; they are substantially faster and have a 10% greater yield than solid media. In 2007, these systems were recommended by WHO to be used in combination with antigen-based species confirmation for diagnosis and drug susceptibility testing (DST) in low-income and middle-income countries.[82] However, such systems are expensive and prone to contamination. Alternative inexpensive non-commercial culture and DST methods were endorsed by WHO in 2009 for use as an interim solution in resource-constrained settings.[81] These alternatives include microscopically observed drug susceptibility (MODS) and the nitrate reductase assay.[78]

To enhance capacity for rapid diagnosis of MDR tuberculosis, WHO in 2008 approved the use of line probe assays (LPAs) for the rapid molecular detection of drug resistance in smear-positive specimens or culture isolates.[82] Two commercial LPAs have shown high accuracy when applied to culture isolates and one of these, the GenoType MTBDR*plus* assay (Hain Lifescience GmbH, Nehren, Germany), also shows very good performance characteristics when applied directly to smear-positive sputum specimens.[83,84] In 2009, the GenoType MTBDRsl assay became available which is also able to detect resistance to fluoroquinolones, aminoglycosides, and ethambutol in culture isolates or smear-positive sputum specimens.[85] When used in combination with the GenoType MTBDR*plus* assay, this potentially provides a means of rapid detection of XDR tuberculosis. These and similar molecular assays reduce the time to diagnosis of MDR and XDR tuberculosis from weeks or months to a matter of days. However, it has yet to be shown whether the use of such assays improves patient outcomes.

Although commercially available, serological tests for tuberculosis are of little diagnostic value,[78] mycobacterial antigen detection is theoretically more attractive, overcoming many of the limitations inherent to

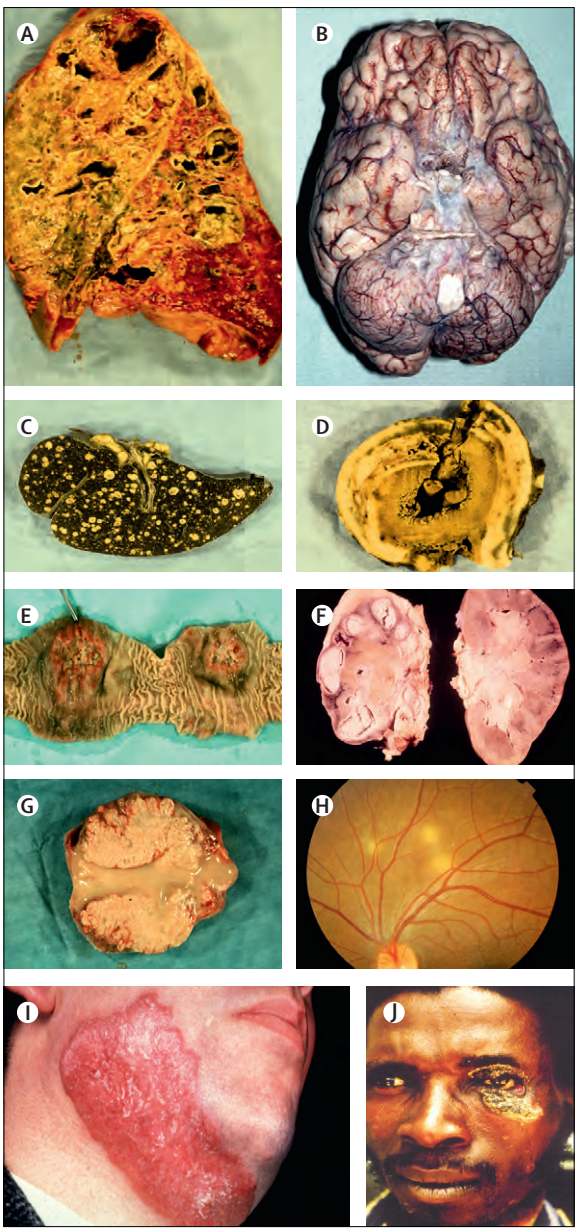

Figure 6: **Tuberculosis in various organs**
Lung specimen showing several cavities, caseous necrosis, and extensive lung pathology due to tuberculosis (A). Ventral view of a brain specimen showing basal tuberculous meningitis and a fibrinous meningeal exudate (B). Multiple caseating lesions of miliary tuberculosis in the spleen (C). Cross-section of the heart showing chronic tuberculous pericarditis with a dense 2 cm thick band of fibrosis (D). Caseating tuberculous ulcers of the ileum (E). Kidney specimens showing caseating lesions due to tuberculosis (F). Section of lymph node showing florid caseating tuberculous lesions (G). Eye fundus with miliary choroid tubercles in an HIV-infected patient with miliary tuberculosis (H; photograph provided by Miles Stanford, King's College London, London, UK). Chronic granulomatous lesions of the face due to *Mycobacterium tuberculosis* (lupus vulgaris; I and J). Photographs (except that of the eye) provided by Sebastian Lucas, St Thomas's Hospital, London, UK.

immune-based assays. A simple commercially available assay is able to detect lipoarabinomannan excreted in the urine of patients with tuberculosis. Although the sensitivity has been disappointing in patients not infected with HIV, moderate sensitivity and high specificity has been noted in patients infected with HIV with advanced immunodeficiency[86,87] and point-of-care versions of this assay are being assessed.[88]

Nucleic acid amplification tests (NAATs) are the most promising development in tuberculosis diagnostics.[78] In the USA and Europe, these tests have been shown to have high specificity, but slight and variable sensitivity, especially for sputum smear-negative disease.[89,90] Simplified versions of these assays with higher sensitivity are being developed. A simplified manual NAAT that uses loop-mediated isothermal amplification with a simple visual colorimetric readout,[91] is being assessed in peripheral laboratory facilities in resource-constrained settings.

A sensitive and specific fully automated and commercially available NAAT assay has now been developed for use outside reference laboratory centres.[92] This Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) uses a series of molecular probes and real-time PCR technology to detect *M tuberculosis* and the *rpoB* rifampicin resistance mutation. The cartridge-based system dispenses with the need for the sputum to be processed in advance, needs minimum laboratory expertise, and results are available in less than 2 h, which permits a specific tuberculosis diagnosis and rapid detection of rifampicin resistance. A large multicountry assessment[93] found excellent performance characteristics. In culture-positive patients, a single direct Xpert MTB/RIF assay identified 551 (98%) of 561 patients with sputum smear-positive tuberculosis and sensitivities were 72·5% when processing one sputum specimen, 85·1% when processing two, and 90·2% when processing three for sputum smear-negative disease.[93] Specificity was 99·2%. If these results are replicated under field conditions at points of care, and the price of introducing the Xpert MTB/RIF assay to points of care in resource-poor countries is brought down, it will represent a major breakthrough in rapid tuberculosis diagnostics and for rifampicin resistance screening.

For the past century, the tuberculin skin test has been the only screen available for the diagnosis of latent infection with tuberculosis. Its major failing is its inability to reliably distinguish individuals infected with *M tuberculosis* from individuals sensitised to other mycobacteria, including BCG. A decade ago the interferon-γ release assays (IGRAs) were developed whereby interferon-γ titres were measured after in-vitro stimulation of peripheral blood mononuclear cells with antigens such as ESAT-6 and CFP-10 (immunodominant antigens expressed by members of the *M tuberculosis* complex).[94] These have now become the gold standard for identifying individuals whose immune system has previously encountered *M tuberculosis*.

Two commercial methods were introduced—the T-SPOT.TB test (Oxford Immunotech, Abingdon, UK) and the QuantiFERON-TB Gold in tube (Cellestis Ltd, Carnegie, Australia)—and have been extensively tested in many clinical situations and in individuals infected with HIV.[94]

The assessment of these tests' results for detection of latent tuberculosis have been difficult because of the absence of a gold standard for tuberculosis latency. A meta-analysis of these studies showed that IGRAs are at least as sensitive and more specific than the tuberculin skin test.[94] Longitudinal studies have shown that the predictive value of IGRAs for reactivation of tuberculosis in immunosuppressed individuals is better than that provided by the tuberculin skin test in individuals vaccinated with BCG.

High levels of interferon-γ release are detected by these assays in about 70–90% of individuals with active disease[94] and these levels decrease after treatment is completed, although such reductions are not consistently recorded.[95–97] The high sensitivities but low specificities of the IGRAs noted in several phase 2 tuberculosis diagnostic studies suggest that IGRAs can be used as rule out but not rule in tests for diagnosis of active tuberculosis.[94,98,99] Further studies of unselected patients need to be done in controlled trials. More recent phase 2 studies of other markers of T-cell responses have shown that interferon γ-inducible protein 10, interleukin 10, and monocyte chemotactic protein 1, show potential for improved detection of active tuberculosis in patients.[100]

Clinical presentation

Although tuberculosis predominantly affects the lung, it can cause disease in any organ (figure 6) and must be included within the differential diagnosis of a vast range of clinical presentations. Symptoms and signs include those associated with the specific disease site as well as non-specific constitutional symptoms such as fever, weight loss, and night sweats. However, in the early stages of disease, symptoms might be absent as shown by community-based active case finding studies in Asia[101] in which about one in four culture-confirmed cases of pulmonary tuberculosis were reported to be asymptomatic.

A high index of suspicion for tuberculosis must especially be maintained when caring for patients living with HIV infection, since risk of tuberculosis is high and diagnosis is difficult. Although patients with high CD4 cell counts present with typical features of tuberculosis, progressive immunodeficiency substantially affects the spectrum of disease with increasing risk of extrapulmonary and disseminated disease.[102,103] Conventional screening for cough lasting 2 or 3 weeks typically has a sensitivity of less than 50% for active tuberculosis in this patient group and about 20% of patients detected on active screening report no symptoms at all.[104] Active microbiological screening for tuberculosis irrespective of symptoms is therefore an important approach and might detect a substantial burden of disease. Up to 25% of patients screened before starting antiretroviral drugs in South Africa have sputum-culture positive tuberculosis.[86,105] Clinical samples from extrapulmonary sites such as fine needle aspiration biopsy of lymph nodes might also provide incremental diagnostic yield.[106]

***Panel 1:* Recommendations included in the WHO guidelines for the treatment of drug sensitive tuberculosis**

First-line 6 month treatment regimen

New patients with pulmonary tuberculosis should receive a regimen containing 6 months of rifampicin: 2HRZE/4HR (high grade of evidence)

Alternative first-line continuation phase

In populations with known or suspected high levels of isoniazid resistance, new tuberculosis patients can receive HRE as treatment in the continuation phase as an acceptable alternative to HR (weak evidence)

Optimum dose frequency

- Wherever feasible, the optimum dose frequency for new patients with pulmonary tuberculosis is daily throughout the course (high grade of evidence)
- Alternatively, new patients with pulmonary tuberculosis can receive a daily intensive phase and then three-times weekly continuation phase provided that each dose is directly observed: $2HRZE/4(HR)_3$ (high/moderate grade of evidence)
- Alternatively, new patients with pulmonary tuberculosis can receive three-times weekly dosing throughout treatment, provided that every dose is directly observed and the patient is not living with HIV or living in a high HIV prevalence setting: $2(HRZE)_3/4(HR)_3$ (high/moderate grade of evidence)

Retreatment regimens and detection and treatment of drug resistance

- Ideally, DST is done for all patients at the start of treatment, so that the most appropriate treatment for each individual can be established
- Specimens for culture and DST should be obtained from all previously treated patients with tuberculosis at or before the start of treatment; DST should be done at least for isoniazid and rifampicin
- In settings where rapid molecular DST is available, the results should guide the choice of regimen
- In settings where rapid molecular-based DST results are not routinely available to guide the management of individual patients, empirical treatment should be started as follows:
  - Tuberculosis patients whose treatment has failed or other patient groups with high likelihood of MDR tuberculosis should be started on an empirical MDR regimen
  - Tuberculosis patients returning after defaulting or relapsing from their first treatment course can receive the retreatment regimen containing first-line drugs 2HRZES/1HRZE/5HRE if country-specific data show low or medium levels of MDR in these patients or if such data are not available
- In settings where DST results are not yet routinely available to guide the management of individual patients, the empirical regimens will continue throughout the course of treatment

Numbers in the treatment algorithms are the months of treatment and subscript number is the dose frequency per week. H=isoniazid. R=rifampicin. Z=pyrazinamide. E=ethambutol. DST=drug susceptibility testing. MDR=multidrug resistant.

The initial clinical presentation of patients with drug-resistant *M tuberculosis* strains does not typically differ from that of patients with resistant strains. Drug-resistant tuberculosis should be suspected in patients who do not respond to the intensive phase of standard short course therapy, have previously had tuberculosis, have a history of poor adherence aggravated by social deprivation or substance abuse, are known contacts of patients with drug-resistant tuberculosis, or who live in regions where the prevalence of drug-resistance is known to be high. However, more than half of the patients with drug-resistant tuberculosis have none of these risk factors and

Panel 2: **Principles underlying the treatment of multidrug-resistant tuberculosis adapted from WHO guidelines**

Number of drugs
Treatment regimens should consist of at least four drugs with either certain, or almost certain, effectiveness. Often more than four drugs are started if the susceptibility pattern is unknown or questionable.

Reliability of DST
In general, susceptibility testing for isoniazid, rifampicin, the fluoroquinolones, and the injectable drugs is fairly reliable. For other drugs this is less reliable and basing individualised treatments on DST for these drugs should be avoided.

Treatment administration
Each dose of an MDR regimen should be given by directly observed therapy throughout the treatment.

Monitoring treatment response
To assess treatment response, smears and cultures should be done monthly until smear and culture conversion (two negative smears and cultures taken 30 days apart). Thereafter smears should be monitored at least monthly and cultures quarterly.

Duration of intensive phase
The intensive phase of treatment for MDR tuberculosis is defined by the duration of treatment with the injectable drug. This should be given for a minimum of 6 months and for at least 4 months after the patient first becomes and remains smear or culture negative.

Total duration of therapy
Treatment for MDR tuberculosis should be given for a minimum of 18 months after culture conversion, but extension to 24 months might be indicated in patients with chronic disease with extensive pulmonary damage.

DST=drug susceptibility testing. MDR=multidrug resistant.

thus ideally every patient with proven tuberculosis should be tested for drug susceptibility.

Treatment

The WHO revised international guidelines for the treatment of tuberculosis in 2010,[107] specifically responding to the growing evidence base[108–111] and escalating problem of drug-resistant disease worldwide.[32] Earlier guidelines emphasised the use of two main standardised treatment regimens, one for new (previously untreated) cases and one for patients with sputum smear-positive disease who had previously received treatment (retreatment regimen). The drug combinations used in these two regimens differed only by the addition of a single drug—a far from optimum situation with regard to prevention of emergence of drug resistance. Lack of laboratory infrastructure for culture and DST in many settings with a high burden of tuberculosis resulted in widespread empirical use of the retreatment regimen. Blind therapy combined with intermittent treatment adherence might have inadvertently fuelled the emergence of multidrug-resistant strains.[112]

Panel 1 summarises the key recommendations from the 2010 WHO tuberculosis treatment guidelines.[107] Rifampicin should now always be given throughout the total 6 months duration of the first-line regimen. New emphasis is placed on the crucial role of DST for guiding the individual management of patients who have previously received treatment for tuberculosis. At present infrastructure for routine DST is scarce, precluding widespread adoption of this recommendation. For settings where this is not routinely possible, national tuberculosis programmes are strongly encouraged to assess country-specific drug resistance data for patients with treatment failure, relapse, and default to inform local policy on empirical use of either retreatment or MDR tuberculosis treatment regimens. Recommendations for the management of drug-resistant tuberculosis are contained within the WHO 2010 tuberculosis treatment guidelines.[107] Panel 2 summarises key principles of treatment, which need carefully informed choices of optimum drug combinations.[113]

Treatment for drug-resistant disease is very costly and prolonged, and is associated with high rates of drug-related toxic effects. However, successful outcomes for MDR tuberculosis are achievable in about two-thirds of patients.[114] Outcomes of treatment for XDR tuberculosis are very heterogeneous;[114–117] although HIV-negative patients in Peru had similar outcomes to those of patients with MDR tuberculosis,[117] XDR tuberculosis was almost universally fatal in a localised outbreak in patients infected with HIV with advanced immunodeficiency in South Africa.[116] In most parts of the world, access to such therapy is very poor, with less than 2% of patients with MDR tuberculosis worldwide treated according to WHO standards in 2008.[2] Thus, in May, 2009, the World Health Assembly passed a resolution urging member states to provide universal access to diagnosis and treatment of MDR and XDR tuberculosis.[32]

Although patients with HIV-associated tuberculosis receive the same antituberculosis treatment regimens as patients not infected with HIV, an additional package of care added to their management regimen can reduce their high mortality risk.[118] HIV testing serves as the crucial gateway for accessing such care and yet only 26% of tuberculosis patients worldwide were tested in 2009.[4] Provider-initiated counselling and HIV testing can greatly increase access to appropriate care[119,120] and rates of testing reached 53% in sub-Saharan Africa in 2009.[4] Mortality risk is reduced by 22–48% with the use of co-trimoxazole prophylaxis[121] and by 64–95% with antiretroviral therapy (ART).[122]

Data are now emerging from controlled clinical trials of the optimum time to start ART in such patients.[123] These studies have shown that irrespective of the CD4 cell count, deferral of ART to the end of treatment for tuberculosis is associated with high mortality risk[124] and

that mortality is reduced by 34% in patients in Cambodia with very low CD4 cell counts (median 25 cells per μL) who are started on ART within the first 2 weeks of treatment rather than after 2 months.[125] The 2010 revision of the WHO ART guidelines recommends that, irrespective of CD4 cell count, all patients with HIV-associated tuberculosis should receive ART as soon as possible during the first 2–8 weeks of treatment for tuberculosis.[126]

Despite pharmacokinetic interactions and potential co-toxicity,[127] excellent virological responses and low rates of treatment limiting toxic effects are noted with overlapping efavirenz-based ART and tuberculosis treatment.[118,128,129] However, use of rifampicin in patients that receive protease-inhibitor-based treatment remains more problematic, needing either dose adjustment or preferably substitution of rifampicin with rifabutin where available.[130] Tuberculosis immune reconstitution disease might complicate ART when started during treatment for tuberculosis, with rapid restoration of pathogen-specific immune responses resulting in the deterioration in the clinical characteristics of tuberculosis.[131] A systematic review[132] reported that this complication developed in 16% (95% CI 10–25) of patients. In most patients immune reconstitution disease is self-limiting, but a small proportion (about 3%) of such patients die.[131,132] A randomised controlled trial has shown that morbidity associated with tuberculosis immune reconstitution disease can be effectively reduced with the use of corticosteroids.[133]

Future successes in the control of tuberculosis will depend on the development of new antituberculosis drugs used in treatment regimens that are shorter, easier to deliver, safe, and low in cost. After decades of neglect, ten new drugs for the treatment of tuberculosis are in the clinical development pipeline of which six were specifically developed for tuberculosis.[134] Examples include the diarylquinolone, TMC-207, which targets mycobacterial ATP synthase and in a phase 2 clinical trial greatly increased sputum smear conversion in patients with MDR tuberculosis.[135] Nitroimidazoles, such as PA-824 and OPC-67683, are equally active against drug-susceptible and drug-resistant tuberculosis[136] and are also being assessed in clinical trials. Since nitroimidazoles are active against both replicating and non-replicating organisms, they could potentially shorten treatment of active disease and provide activity against latent infection with tuberculosis.[134] Assessment of the use of moxifloxacin, gatifloxacin, and TMC-207 in treatment regimens to shorten the duration of chemotherapy are ongoing.

Vaccines for tuberculosis

There is a dire need for a universally effective vaccine for the control of tuberculosis.[137,138] The only licensed vaccine, BCG, was first given to a human infant in 1921. The vaccine has been given to 4 billion people so far and to more than 90% of the children in the world today, making it the most widely used vaccine in the world. However, it has done little to contain the current tuberculosis pandemic. Despite evidence of confirmed efficacy against childhood tuberculous meningitis and miliary tuberculosis, protection induced by BCG can wane within a decade and thus the efficacy against adult pulmonary tuberculosis is variable.[139] Current pre-exposure vaccination strategies can only aim at reducing the initial *M tuberculosis* bacterial burden and at preventing reactivation of latent infection. Post-exposure vaccines are also needed to target these dormant bacteria and prevent their reactivation. Post-exposure vaccines would ideally also prevent reinfection of individuals living in regions of high tuberculosis prevalence. Whole heat-killed *Mycobacterium vaccae*, an environmental saprophyte, has been tested as an immunotherapeutic agent for the treatment of tuberculosis in three trials in Africa with discrepant outcomes.[140] A randomised, placebo-controlled, double-blind study of *M vaccae* given in several doses to BCG-vaccinated participants infected with HIV, showed a reduction in cases of tuberculosis.[141] A dozen new vaccine candidates are in clinical trials, with the aim of either replacing the existing BCG vaccine or enhancing immunity induced by BCG.[137] Several phase 1 and 2 clinical trials are underway in South Africa.[112]

Tuberculosis control

After the declaration in 1993 that tuberculosis was a global emergency, WHO launched the directly observed treatment, short-course (DOTS) strategy, which was successfully expanded as the principal tuberculosis control strategy, focusing primarily on detection and effective treatment of infectious cases. Between 1995 and 2008, 43 million people were treated under DOTS, 36 million were cured, case-fatality rates decreased from 8% to 4%, and an estimated 6 million deaths were potentially averted.[2] After a decade of implementation, the new STOP TB Strategy and the Global Plan to Stop TB (2006–15) were launched in 2006 to address important challenges that included the HIV-associated tuberculosis epidemic, the emergence of the MDR tuberculosis epidemic, weak health systems, and insufficient engagement with private health-care providers and with communities.[142,143]

Progress is being monitored against an established series of goals and targets (panel 3).[144] The earlier worldwide target of an 85% treatment success rate for sputum smear-positive cases was first achieved in 2007, and the Millennium Development Goal 6 target to reverse the rising incidence in tuberculosis incidence rates has been fulfilled since 2004.[8] However, although the worldwide case detection rate increased substantially between 1995 and 2008, it has stabilised at around 60%, falling substantially short of the 70% target. Moreover, the targets of halving the 1990 tuberculosis prevalence and mortality rates by 2015 are unlikely to be met worldwide, with the epidemics of HIV-associated tuberculosis in Africa and MDR tuberculosis in eastern Europe being key stumbling blocks.[2]

Panel 3: **Performance targets for tuberculosis control**

World Health Assembly, 1991

Targets originally set for 2000, later postponed to 2005 and now deemed obsolete in view of the call for universal detection and cure:

- Achieve a worldwide case detection rate of 70%
- Achieve a worldwide cure rate of 85%

MDG 6

Target 6.c: halt and begin to reverse the incidence of tuberculosis by 2015

Targets linked to the MDGs and endorsed by the STOP TB Partnership

By 2015: reduce the prevalence of tuberculosis and deaths due to tuberculosis by 50% compared with the baseline of 1990

By 2050: to eliminate tuberculosis as a public health problem as defined by achieving a worldwide incidence of tuberculosis of less than 1 case per million population per year

Adapted from the WHO Global Plan to STOP TB 2011–15.[144] MDG=Millennium Development Goal.

Despite substantial progress in worldwide tuberculosis control, it is unclear why tuberculosis incidence is only falling at a rate of less than 1% per year. The potential benefits of DOTS might be offset by changing population risk factors[145] or late tuberculosis diagnosis might mitigate the effect on transmission rates. Rates of decline might also be more strongly related to social and economic factors and general population health than the performance of national tuberculosis control programmes.[3,146] To eliminate tuberculosis as a public health problem by 2050, incidence must fall by an average of 16% yearly over the next 40 years.[145] However, even if the Global Plan to Stop TB were successfully implemented, incidence would only decrease at around 6% yearly, meaning that worldwide incidence rates in 2050 would remain 100-times higher than the elimination target.[27] In addition to biomedical interventions related to tuberculosis, improvements in tuberculosis control will also need to progress in the development and strengthening of health systems and progress in the broader development agenda.[7,147]

One of the greatest challenges to tuberculosis control is the HIV-associated tuberculosis epidemic, for which the DOTS strategy is insufficient.[5,118] Preventive interventions are also needed and the four principal tuberculosis prevention methods available include intensified case finding, isoniazid preventive therapy (IPT), tuberculosis infection control, and ART.[118] So far ART is the only one of these that has been implemented at scale, provided to an estimated 5·3 million people in low-income and middle-income countries by the end of 2009.[148] By contrast, only 4·1% of HIV-infected patients benefited from intensified tuberculosis case finding and 0·2% of eligible individuals received IPT in 2008.[2] To galvanise greater momentum in the implementation of these interventions, WHO launched in 2008 the 3Is policy, which consists of IPT, intensified case finding, and infection control to be scaled up in parallel with ART.[149,150]

A key reason underlying the failure of DOTS to control the HIV-associated tuberculosis epidemic in Africa is that it fails to address the fundamental epidemiological interactions between tuberculosis and HIV in which progressive immunodeficiency fuels high rates of disease. Immune recovery during ART is associated with a 67% (95% CI 61–73) reduction in tuberculosis incidence rates in cohorts in both high and low tuberculosis burden settings and irrespective of tuberculin skin test status.[122,150] The overall effect at a population level will depend greatly on the time that patients spend at low CD4 cell counts both before and during ART.[150,151] Much more aggressive scale-up of ART in combination with the 3Is interventions would potentially have a greater effect on this epidemic.[152]

A major impediment to achieving control of tuberculosis is the lack of resources to effectively implement the Global Plan to Stop TB. The estimated funding needed for the period 2006–15 was US$60 billion. Funding for tuberculosis control in the 22 high burden countries increased from $1·84 billion in 2006 to an estimated $2·64 billion in 2010.[153] However, during the same period, the projected funding shortfall also increased from $145 million to $500 million. Although donor funds might facilitate the provision of essential tuberculosis and HIV services, local ownership and identification of sustainable local solutions are the key to the control of tuberculosis and for donor-dependent programmes to transfer responsibility to local governments and long-term sustainable funding models. Securing adequate funding to meet the huge demand in the current economic recession, presents a formidable challenge.

Conclusions

Tuberculosis remains a major cause of death and morbidity worldwide, and control efforts so far have not adequately controlled the epidemic in many parts of the world, especially in the countries of sub-Saharan Africa and parts of eastern Europe. Absence of a cheap point of care diagnostic test, the long duration of treatment, lack of an effective vaccine, emergence of drug-resistant tuberculosis, and weak health systems in resource-poor developing countries are all factors that continue to hamper progress towards achieving control of tuberculosis worldwide. Despite this, there is growing momentum in basic and applied research activity that is starting to yield new diagnostic, treatment, and prevention methods, and now provide grounds for optimism. However, this growing scientific momentum must be matched by massive political and funder commitment to provide adequate funding to ensure that the aims of the WHO Global Plan to STOP TB 2006–15 are achieved.[153]

Contributors
SDL and AZ contributed equally to the preparation of this Seminar.

Conflicts of interests
We declare that we have no conflicts of interest.

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