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EUROPEAN RESPIRATORY UPDATE

Update on tuberculosis: TB in the early 21st century

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Mycobacterium tuberculosis infection remains a major cause of global mortality and morbidity and the resulting disease, tuberculosis (TB), caused an estimated 1.7 million deaths in 2009 [1]. However, the majority of the 2 billion people estimated to be infected with *M. tuberculosis* have asymptomatic infection, termed latent TB infection (LTBI) [2, 3]. Traditionally, LTBI has been defined by evidence of a cellular immune response to *M. tuberculosis*-derived antigens, for example by the tuberculin skin test (TST) in asymptomatic individuals who have been potentially exposed to *M. tuberculosis*. For these individuals, the life time risk of progression from LTBI to active, symptomatic disease is only 10%; however, defects in cellular immunity can increase this risk significantly [4, 5]. Of these, the most important is HIV infection, and the ongoing HIV epidemic has helped to fuel the spread of TB globally over the past 40 yrs with 0.38 million deaths in HIV co-infected individuals in 2009 [1].

Despite dramatic falls in the overall prevalence of TB over the past 100 yrs in Europe, TB is no longer a disease of historical interest and the European region defined by the World Health Organization (WHO) saw a 2.7% mean annual increase in cases from 2004–2008 [6]. Of concern, an estimated 3.6% of all new cases globally were multi-drug resistant (MDR) [1, 7].

With this in mind, we present an update on a selection of important areas in TB clinical practice and clinical research today, with particular relevance to Europe. We will review some of the important epidemiological features of TB, with a focus on migration and recent work on factors which might influence the burden of TB at a population level, such as cigarette smoking/biomass fuel use and vitamin D deficiency. We will also present an update on important developments in TB diagnostics over the past decade, in both latent infection and active disease, briefly discuss TB/HIV and review advances in the treatment of TB, including the management of drug-resistant disease.

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UPDATE ON THE EPIDEMIOLOGY OF TB INFECTION

In 2008, the WHO European region accounted for ~6% of all new cases of active TB disease globally, reporting 461,645 cases; 11.1% of these were MDR [6]. The overall notification rate was 16.7/100,000, but this reflects great variation within the region, from 1.9/100,000 in Iceland to 115.1/100,000 in Romania. Moreover, significant variation occurs within countries, with most cases concentrated within urban centres such as London (UK), which accounted for ~38% of the total number in the UK in 2009 [8]. Globally, the incidence of TB fell in 2009, but population growth has offset this decrease so that the absolute number of new cases continues to increase [9].

The global plan to stop TB

Thus, at the beginning of the 21st century, the absolute number of new incident cases of TB continues to rise, despite the fact that TB is a treatable and curable illness. To try and close this gap between what is being done and what could be done, a coalition of partners, including the WHO, established the Stop TB Partnership in 2001. Through its global plan to stop TB, its goal is to control and eliminate TB as a global public health problem and in doing so has focussed on seven discrete areas, formulated as working groups: DOTS (directly observed treatment, short course) expansion, TB/HIV, MDR-TB, new TB drugs, new TB vaccines, new TB diagnostics, and advocacy, communications and social mobilisation. In re-formulating its strategy in 2005 it set a key target that by 2015 the global burden of TB disease will be reduced by 50% relative to 1990 levels [10]. The financial, social and political will needed to achieve this is significant: an estimated 56 billion US$ is required for implementation of the plan in 2006–2015, and the funding gap for this is significant. However, with coordinated action, the will of governments and donors to help meet these targets has been strengthened. It may be that of all the developments in TB science and research so far this century, creation of the global plan and the Stop TB partnership is one of the most important.

TB and migration

Human migration is significant socially, financially and medically and there are currently ~214 million people living outside their country of birth and 16.3 million refugees in the world today; the annual migration growth rate is estimated to be 2.9% [11]. The reasons for migration are diverse and include economic, socio-political and climatic push and pull factors.

Migration is one of the key epidemiological determinants of TB in low-burden European countries: the majority of new diagnoses of active TB disease are due to foreign born persons.

For example, in the UK in 2009, non-UK born individuals accounted for 73% of new cases [8]. However, most cases of TB in low to intermediate incidence European countries are due to reactivation of LTBI, with low rates of transmission of *M. tuberculosis* from foreign born persons to native born persons [12–18]. Nonetheless, the financial consequences of TB in migrated individuals in terms of medical expenditure are still significant, particularly in those with MDR-TB [19].

Since most cases of active disease arise within the first few years following immigration [20], as a result of reactivation of LTBI [12–18], reducing the burden of TB in low-burden countries will require improved identification of latently infected individuals and the use of appropriate preventative treatment. Chemoprophylaxis, with 6–9 months of isoniazid or 3 months of a combination of rifampicin and isoniazid, can reduce the rates of progression to active TB by ~60% [21, 22]. Thus, understanding the dynamics and interaction of TB and migration to low-incidence countries is important, as is developing cost-effective and practically useful strategies to screen migrants for TB.

Appropriate screening programmes for migrants from high-burden countries are therefore a key component of strategies to reduce the number of new infectious cases in low-burden countries, although controversy exists on how best to approach this practically [19, 23–26]. Nationally, and within countries, differences exist on the site of screening (port of origin *versus* port of arrival *versus* primary care) and on the tests performed (chest radiography *versus* TST *versus* interferon (IFN)-γ release assay *versus* a combination). Practice across Europe is variable; in England and Wales for instance, there is a highly variable approach to screening, which does not necessarily follow national guidelines or even local need [27].

Although a systematic appraisal of the use of newer diagnostic tests, including IFN-γ release assays (IGRAs), in screening new arrivals to low-burden countries has not yet been undertaken, the use of an IGRA alone as a screening tool has recently been suggested to be cost-effective in new entrants from high-incidence areas of >200/100,000 cases and may also improve testing strategy uptake when compared with the TST [28, 29].

As noted elsewhere, the most sensible way to reduce the burden of TB amongst migrants from high-burden countries is to reduce the burden of TB in those high-burden countries themselves and better understand the factors which drive migration globally. However, as the number of global migrants will increase in the short to medium term, full implementation of cost-effective screening strategies appropriate to each country's needs are required now to help control and eliminate TB in low-burden, high-income countries.

Modifiable environmental risk factors and TB

A better understanding of those factors which lead to *M. tuberculosis* infection or which increase the risk of progression to active TB disease is also important to aid efforts to control the TB epidemic. Traditional, well-recognised factors which lead to an increased risk of reactivation of LTBI at an individual level include defects in cellular immunity, such as HIV infection, immunosuppression from solid organ and haematological malignancy or from the treatment of these conditions, and immunosuppressive drugs such as tumour necrosis factor (TNF) antagonist agents [30, 31]. More recently, however, research has focussed on other key environmental and social modifiable risk factors, the modification of which at the population level could have significant impact on the incidence of TB [32–34].

Cigarette smoking changes the inflammatory milieu of the lung and alters alveolar macrophage function [35]. A systematic review and meta-analysis of the available literature by BATES *et al.* [36] showed that cigarette smoking increases the risk of both *M. tuberculosis* infection (by up to 70%) and progression to TB disease (by up to 50%). They did not demonstrate any effect of cigarette smoking on mortality from TB, although a prospective cohort study in Taiwan has since shown a favourable effect of smoking cessation on mortality from TB [37]. The use of biomass fuels and indoor cooking has been associated with an increased risk of developing respiratory disease, including TB [38]. The data linking biomass fuel use to TB are more limited than those available for cigarette smoking, and largely come from case–control studies [39, 40]. However, a recent modelling study demonstrated the potentially significant impact that cessation of both cigarette smoking and indoor solid fuel use could have on TB incidence in China [41]. Whether these data are meaningful outside of an Asian setting and translate fully from the model to the real world remains to be seen, but they do suggest that smoking cessation policies could have potential benefits in helping to reduce the global burden of TB.

Other important population factors associated with TB disease include alcohol consumption and diabetes mellitus [42, 43]. The value of screening for TB in patients with diabetes mellitus (and *vice versa*) has been suggested to be of possible benefit in improving the identification of patients with TB, although the cost-effectiveness of this has not been determined [44].

Vitamin D deficiency and TB

There has been increasing interest in a possible link between vitamin D deficiency and TB. In the first half of the 20th century, TB was treated with cod liver oil and phototherapy (for *Lupus vulgaris*); however, it wasn't until the 1980s that it was demonstrated that patients with TB had significantly lower levels of 25-hydroxyvitamin D (25(OH)D) than matched controls [45]. This work was confirmed in a case–control study in London (UK) in patients with active TB and matched healthy TB contacts where a clear gradient of effect was shown to exist [46]. Vitamin D deficiency was shown to be associated with active TB disease and in patients with undetectable 25(OH)D, the effect was greater still. This association is not confined to the UK and a meta-analysis in 2008 confirmed that this effect was not restricted by geography or ethnic group [47]. Importantly, work elucidating the cellular pathways underpinning the links between vitamin D and host defence against *M. tuberculosis* suggests a key role for the anti-bacterial peptide cathelicidin [48, 49].

The effect of vitamin D supplementation has been further explored in three recent randomised controlled trials (RCTs). Each used different methods of vitamin D supplementation and hence may not be directly comparable. The first in Indonesia, showed a positive effect of vitamin D supplementation on time to sputum culture conversion in patients with

active pulmonary TB [50]. The second in Guinea-Bissau, demonstrated no effect using similar outcome measures, although the mean vitamin D concentration at recruitment was within the normal range and patients in the volunteer arm surprisingly incremented their vitamin D during the trial, thus potentially reducing the power of the study [51]. The third study showed that vitamin D therapy could significantly reduce time to sputum culture conversion only in an *a priori* defined subgroup of patients in London, UK with a *Taq*I polymorphism (*tt* genotype) at the vitamin D receptor [52].

It therefore remains to be definitively shown that treatment with vitamin D benefits patients with active TB disease. It may be that vitamin D would be more useful in preventing progression to active TB in LTBI; however, demonstrating this will require extremely large and adequately powered studies.

UPDATE ON DIAGNOSTICS IN LTBI

LTBI is thought to arise following exposure to *M. tuberculosis* when the host's adaptive immune system is able to control but not eliminate the organism, resulting in asymptomatic infection without detectable bacilli or sterilising immunity. However, as bacilli are by definition not detectable in LTBI, the diagnosis currently depends on detecting the host's immune response to the infection. Accurate detection of LTBI is a key strategy of TB elimination strategies in low-incidence countries [53].

For >100 yrs diagnosis of LTBI was based on the TST: the delayed hypersensitivity response to purified protein derivative (PPD; a cocktail of proteins derived from *M. tuberculosis*) injected intradermally. This test is cheap and simple to perform but requires two patient contacts (first to inject the PPD, and secondly to read the result 48–72 h later). In addition, patients sensitised to environmental nontuberculous mycobacteria or patients vaccinated with the bacille Calmette–Guérin (BCG) may have a false positive result. In patients who are immunosuppressed and in children, those most at risk of developing active TB, the test loses sensitivity, potentially resulting in false negative results [54, 55].

Changing the diagnosis of LTBI in the last decade: IGRAs

The development of IGRAs in the past 10 yrs has tried to address some of these difficulties [56]. IGRAs work on the principle of measuring the IFN-γ released from T-cells specific to *M. tuberculosis* as a marker of infection. By using specific protein antigens secreted from *M. tuberculosis* encoded in the region of difference 1, which is not present in the BCG or in the majority of nontuberculous mycobacteria, they are theoretically more specific than the TST. IGRAs exist in two formats: the ELISpot-based IGRA, where individual IFN-γ producing T-cells responding to *M. tuberculosis* antigens are counted, and the ELISA-based IGRA, where the IFN-γ itself is measured in a whole blood assay after stimulation with the antigens. There are currently two commercially available preparations: the ELISpot-based T-SPOT^{TM}.TB (Oxford Immunotec, Abingdon, UK) and the ELISA-based QuantiFERON^{TM}-TB Gold In-Tube (Cellestis, Carnegie, Australia). The first proof-of-principle work demonstrating their potential usefulness was in comparing patients with active TB disease and healthy BCG vaccinated volunteers with low epidemiological risk of TB infection. These early studies showed that (in comparison with patients with culture confirmed TB, the gold standard) the assays were sensitive and importantly not confounded by prior BCG vaccination [57, 58]. In systematically reviewing this work, PAI *et al.* [59] have found the sensitivity of the ELISpot-based approach to be 90% (range 83–100%) and the ELISA-based approach to be 70% (range 64–93%). The pooled specificities of both in this review were >93%.

The evidence supporting their role in the diagnosis of LTBI has been extensively reviewed and will be briefly summarised here [56, 60]. As there is no gold standard for the diagnosis of LTBI (as no bacilli are by definition culturable) surrogate markers have been used to assess the sensitivity of IGRAs in this respect. As infection with LTBI is determined by duration and closeness of contact with the index case, contact tracing studies have been used to compare the performance of these platforms with the TST. In summary, these studies with both ELISpot and ELISA platforms show that IGRAs correlate better with TB exposure than the TST and are not confounded by prior BCG vaccination [61]. The majority of these studies have been in low-burden countries, where remotely acquired *M. tuberculosis* infection is less likely to confound the results; however, these studies have now been successfully replicated in high-burden countries [62] and more recently in a high-burden country with high rates of HIV infection, with similar results [63].

Unlike the TST, IGRAs, particularly the ELISpot-based test, appear to perform reasonably well in patients with defects in cellular immunity. The majority of this evidence comes from studies on children [62, 64] or in patients with HIV [63, 65]. There is also some evidence in smaller studies in patients with autoimmune conditions [66] and in patients with chronic kidney disease [67–70] that the IGRAs remain more sensitive than the TST. This latter group is particularly important, as individuals with LTBI and chronic kidney disease have a much higher risk of progression to active TB [71]. The performance of the ELISA-based test in HIV-infected individuals appears to be poorer, with reduced sensitivity in patients with lower CD4 counts [72–74].

A key development in the treatment of patients with inflammatory conditions such as rheumatoid arthritis and psoriasis has been the development of TNF antagonist therapies, in the form of monoclonal antibodies or soluble receptor antagonists. Perhaps unsurprisingly, given the key role TNF-α has in the host response to *M. tuberculosis*, particularly in granuloma formation and stability [75], individuals treated with these therapies are at significantly higher risk of reactivating LTBI. This increased risk may be up to 25 times higher, depending on the type of drug used [31]. Given this increased risk, all patients with LTBI who are going to receive a TNF antagonist therapy should receive chemoprophylaxis. The background to this topic and the use of IGRAs and TST in the screening of patients with chronic inflammatory conditions has been well-summarised in a recent European Respiratory Society consensus statement [31]. They recommend either the use of IGRAs or TST in patients without BCG vaccination for screening and note that in the paediatric population both strategies are used. Given this, and the high risks associated with a failure to identify LTBI in these individuals, our personal practice is to give chemoprophylaxis to any patient with either a positive TST or a positive IGRA.

In the absence of a gold standard for the diagnosis of LTBI, arguably the best indicator of true LTBI is the subsequent development of active TB disease. A good test for LTBI in this respect will therefore predict who progresses to active TB disease and hence allow the better targeting of appropriate chemoprophylaxis. As only 5–10% of immunocompetent individuals will do this, large numbers of patients need to be tested in order to have a study of sufficient power to test this hypothesis. As a result, such evidence of longitudinal progression in patients who are IGRA positive is more limited but the work that has been performed in low-incidence countries suggest that the IGRAs may predict progression to active disease at least as well as the TST [76–79]. One study in the Gambia did not replicate this finding, although this may relate to its high-burden setting [80].

From this work, a significant number of national guidelines in low-burden countries have now incorporated the use of IGRAs in algorithms for the diagnosis of LTBI. Variation exists on whether this utilises them in place of the TST, as in the USA [81], or alongside the TST, as in the UK [82] and elsewhere in Europe. As more longitudinal data emerges on the cost-effectiveness of different screening strategies, these algorithms are likely to be refined over time.

UPDATE ON IMPROVING THE DIAGNOSIS OF ACTIVE TB DISEASE

The gold standard for the diagnosis of active TB disease is the culture of *M. tuberculosis* from tissue or fluid from the affected area. This can take up to 6 weeks, during which time the clinician must either make the decision to treat the patient empirically, based on clinical and radiological features and smear microscopy if the patient is smear positive, or observe the patient until culture results are available. Given that approximately only 50% of adult patients are smear positive [1] (with a correspondingly lower figure in children) and that the rates of extrapulmonary disease appear to be increasing in some low-incidence European countries [83], the former strategy exposes a number of patients without TB to the risks of antituberculous drugs, and the latter strategy encourages disease progression in the patient with TB, increasing their infectivity over time. Interrupting this cycle of infection is key to reducing the global burden of disease.

Improving the detection and diagnosis of active TB disease is therefore a priority to clinicians in both high- and low-burden countries; doing so will facilitate TB control strategies which aim to reduce the incidence of new infections with *M. tuberculosis* by identifying sources of infection as rapidly as possible [53]. Herein, we present reports on some of the advances in this area over the past decade.

Improving immune-based tests: IGRAs in active disease

Whilst IGRAs have been a significant step forward in the immunodiagnosis of TB, their sensitivity, while better than the TST, is insufficiently high to use them as a rule out test for active TB. DOSANJH *et al.* [84] added further *M. tuberculosis*-specific antigens to improve the sensitivity of the ELISpot platform from 85% to 89% for the diagnosis of active TB in 194 adults. Combining these ELISpot^{PLUS} results with TST results further increased the sensitivity of active TB diagnosis to 99% (95% CI 95–100%), giving a negative likelihood ratio of 0.02 (95% CI 0–0.06) when both test results were negative. This work provides promise that similar approaches may improve sensitivity sufficiently to use these tests, albeit perhaps in combination, as a genuine rule-out test for TB, at least in HIV-negative individuals.

Another approach to increase the sensitivity of IGRAs is to measure alternative cytokines than IFN-γ. RUHWALD *et al.* [85] have used IFN-γ inducible protein 10 in an ELISA platform similar to the existing QuantiFERON^{TM}-TB Gold In-Tube. Their results so far indicate that this approach has potential for improving IGRA test performance, but more data is needed to see how they compare against traditional IGRAs in similar studies to those performed previously.

As discussed, *M. tuberculosis*-specific T-cell responses in whole blood or peripheral blood mononuclear cells (PBMC) are not specific for TB disease and are instead a marker for TB infection. It has been hypothesised that using IGRAs at the site of disease, where *M. tuberculosis*-specific T-cells are concentrated, might be used as a more sensitive and specific marker of TB disease at that body site [86].

The majority of research in this area has attempted to quantitate *M. tuberculosis*-specific pulmonary T-cell responses from bronchoalveolar lavage (BAL) fluid (using an ELISpot platform) [87–89], although significant work has also been performed on pleural [86, 90] and meningeal [91, 92] T-cell responses in active TB. In a large, multicentre European study, JAFARI *et al.* [88] found the sensitivity (compared to a gold standard of culture positivity) of a BAL ELISpot test to be 91% in patients with sputum acid-fast bacilli smear negative TB, superior to both the PBMC ELISpot, the TST and BAL nucleic acid amplification tests. Intriguingly, the specificity of the BAL ELISpot for active TB was only 80%, indicating a significant number of patients with apparent LTBI had *M. tuberculosis*-specific pulmonary T-cells in their lavage fluid. While the sensitivity is probably not high enough to use the test as a rule-out test, this lack of specificity also makes it difficult to use as a rule-in test, and also raises questions about the nature of pulmonary T-cell responses in apparently latent infection. In a high-burden setting, DHEDA *et al.* [89] found that ELISpot responses in alveolar cells were a useful immunodiagnostic test for pulmonary TB, and in fact superior to BAL smear microscopy in cases with definite TB. The positive predictive value of the test was 89% and the negative predictive value was 94%. Interestingly, the specificity of the test was much higher than in the studies from low-burden settings; this may relate to the more advanced stage of disease in this high-burden cohort, 29% of whom were infected with HIV. Of note, and in contrast to JAFARI *et al.* [88] where the cut-offs used were different, 34% of all ELISpot tests in this prospective study were inconclusive, calling into question its clinical utility in this setting.

Pulmonary T-cell responses have also been assessed in induced sputum, a useful technique which is safe, low cost and does not require the training or risks that bronchoscopy entails. Its utility in determining pulmonary T-cell responses has also been examined: CASHMORE *et al.* [93] found that the number of inconclusive results (using an ELISpot platform), resulting from both practical and technical failures made it a

non-clinically useful approach. In contrast, BREEN *et al.* [94], using a technically more demanding flow-cytometric approach and cells stimulated with PPD, were able to diagnose 24 out of 27 patients ultimately diagnosed with active TB. Three out of 15 patients without TB were also found to have positive pulmonary T-cell responses, similar to the proportion found by JAFARI *et al.* [88] using BAL cells and an ELISpot platform. For now, these approaches are of interest but the number of indeterminate results and the technically demanding nature of the assays make them currently difficult to use in a routine clinical setting.

M. tuberculosis-specific T-cell responses have also been found to be present in cerebrospinal fluid and pleural fluid. A growing body of work suggests that these may be helpful in the diagnosis of TB at these sites where traditionally smear negative and culture negative disease is more common. This ''site of disease'' IGRA research has recently been reviewed by SESTER *et al.* [95] in a meta-analysis of the available data. They found that the sensitivity of the ELISpot for the diagnosis of TB was increased from 81% in blood to 88% in extrasanguinous fluid, while the sensitivity for the ELISA fell from 80% in blood to 48% in extrasanguinous fluid. The specificity also improved from 59% to 82% using the ELISpot, which was similar to the ELISA, whose specificity differed little between blood and extrasanguinous fluid (79% and 82%, respectively). Thus, while the concept of using IGRAs in extrasanguinous fluid is an attractive prospect for the diagnosis of active TB, they are neither sufficiently sensitive nor specific to be used in this way at present in routine practice.

Nucleic acid amplification tests

One of the main barriers to the effective global control of TB is the fact that the majority of people with active TB disease live in parts of the world with limited laboratory systems, making accurate diagnosis of TB difficult. Moreover, the rise of drug-resistant disease has not been matched by upscaling of laboratory infrastructures able to diagnose it. For these reasons, there is an urgent need to improve near patient testing for active TB disease in low-income countries.

The original point of care test for TB is sputum smear microscopy: globally it is the most important and frequently used test to diagnose TB. However, this test suffers from a lack of sensitivity, ranging from 32% to 97% depending on the study [96], requires basic laboratory facilities and staff training and does not diagnose drug-resistant disease or extrapulmonary disease. The higher rates of sputum smear negative disease in children and HIV-infected patients, both of whom are disproportionately affected by TB in low-income countries, further reduce its utility as an ideal point of care test. From this background, the Stop TB Partnership Working Group on New TB Diagnostics has called for the development of accurate cost-effective tests to diagnose TB and determine drug resistance [97].

The decoding of the genome of *M. tuberculosis* heralded the beginning of molecular techniques in the diagnosis of TB [98, 99]. Commercially available nucleic acid amplification tests (NAAT) for the diagnosis of TB are specific and sensitive in smear-positive disease, but perform less well in smear-negative disease and require moderately advanced laboratory level facilities and are thus not practical for use in low-income countries. Similarly, line probe assays for the diagnosis of drug resistance such as Genotype MTBDRplus (Hain Lifescience, Nehren, Germany) improve time to diagnosis of drug resistance against first-line drugs, but again require training and facilities beyond the reach of most low-income countries.

In 2010, the evaluation of a novel molecular platform, Xpert MTB/RIF, was reported in 1,730 patients with suspected pulmonary TB in low- to middle-income countries [100]. Importantly, it is a self-contained cassette-based test which does not require intensive training or advanced laboratory facilities and gives results in ~2 h. BOEHME *et al.* [100] showed that it was highly specific and diagnosed 98.2% of smear positive patients and 72.5% of smear negative, culture positive patients (which increased to 90.2% if three tests were used). Crucially, it correctly identified 97.6% of rifampicin-resistant bacilli. This holds considerable potential and could potentially even replace standard smear microscopy; however, further evaluations and reports on its use in real-time clinical practice are needed. Nonetheless, these data have proved sufficiently compelling for the WHO to recommend this test as the first-line test in individuals suspected of having MDR-TB or HIV-associated TB and as a follow-on test for smear negative samples in other patients [101].

Other immunodiagnostic approaches

IGRAs have transformed our ability to more accurately diagnose LTBI. However, similarly to the TST, they simply detect the presence of a host response to *M. tuberculosis* antigens and have not been able to discriminate LTBI from active disease. Therefore, a key goal for researchers in TB immunodiagnosis is to find biomarkers able to differentiate different disease stages in TB, which would help target appropriate therapy, identify when treatment is not working or a patient has relapsed and possibly also provide surrogate markers of vaccine efficacy [102, 103].

One potential approach is the measurement of so-called ''multifunctional CD4 T-cells''. These cells, when phenotyped according to their cell surface markers and cytokine production, have been associated with disease control in other chronic infections such as HIV [104]. Relative changes in the numbers of these cells has been shown to be associated with bacillary load in active TB and their use in differentiating LTBI from active disease is being explored and shows promise [105, 106].

Several studies have attempted to use changes in the expression of host genes in blood cells from patients with TB to derive a gene ''signature'' specific to TB, which could be used to develop a diagnostic test and perhaps differentiate disease stages. MISTRY *et al.* [107] studied patients in South Africa and derived a set of four genes which could discriminate patients with active TB from those with latent TB and those who had been previously treated for TB. Similarly, JACOBSEN *et al.* [108] identified a set of three distinct genes to discriminate patients with active TB from healthy individuals or those with LTBI. A later study based in South Africa attempted to validate this same three-gene set as part of a wider microarray based approach [109]. Although the authors concluded that the three genes could not discriminate between all groups in this separate population, they were able to generate separate microarray profiles discriminating active

TB from healthy individuals and those with latent infection, although these profiles could not discriminate LTBI from healthy individuals. Finally, BERRY *et al.* [110] used a microarray based approach to identify differential gene expression in active and latent TB, identifying a 393-gene signature which discriminated active TB from healthy individuals and those with latent infection, in separate cohorts of patients from both the UK and South Africa, although again these profiles could not discriminate LTBI from healthy individuals. This 393-gene signature also reflected the extent of radiographic disease and was abolished by successful TB treatment. A separate 86-gene signature was able to differentiate patients with active TB not only from those with LTBI, but also from those with other inflammatory and infectious diseases. Interestingly, 10% of their latently infected individuals appeared to have an active disease signature, increasing the possibility that this approach could be used in future studies to attempt to identify those most at risk of developing active disease.

An alternative method is to examine gene expression in cells which are first stimulated with *M. tuberculosis* specific antigens. WU *et al.* [111] were able to differentiate latently infected individuals from patients with active TB by measuring the expression of three genes; however, no healthy individuals were included in this study as a comparator group. Similarly, measuring the ratio of the expression of levels of interleukin (IL)-4 and its splice variant IL-4d2 has been shown to correlate with extent of disease and changes in the ratio may point to changes in bacillary burden [112, 113].

Improving pathogen acquisition and localisation

As noted previously, extrapulmonary TB, particularly intrathoracic lymph node TB, is becoming increasingly common in low-burden countries, the reasons for which are unclear. However, this form of the disease can be difficult to diagnose both clinically and microbiologically given its nonspecific symptoms, low yield on sputum smear examination and that the site of disease may be anatomically less accessible. In suspected tuberculous mediastinal lymph node disease without lung parenchymal involvement, sampling of the lymph nodes *via* mediastinoscopy or thoracotomy was previously necessary to clarify diagnosis, obtain tissue for drug sensitivity testing and rule out other pathologies including benign conditions, such as sarcoidosis and malignant conditions such as lymphoma. Facilitating the diagnosis of this form of the disease is therefore important. Transbronchial needle aspiration (TBNA) *via* a fibreoptic bronchoscope offers a minimally invasive method of lymph node aspiration. Its use, however, is limited to large lymph nodes in well-described nodal stations in most situations, and experience in non-malignant disease has been centred around sarcoidosis [114] with only a small number of published studies describing its use in TB [115, 116]. However, the more recent development of endobronchial ultrasound allows real-time visualisation of mediastinal structures and in combination with TBNA (endobronchial ultrasound-guided (EBUS)-TBNA), a safe method of ultrasound-guided aspiration of smaller mediastinal and hilar lymph nodes [117]. Its main utility has been in the staging of thoracic malignancy [118] but its use is also well described in benign disease such as sarcoidosis [119, 120]. Data is now emerging that EBUS-TBNA is a safe and useful modality for the diagnosis of intrathoracic lymph node TB [121, 122] with acceptable culture rates [123]. Similarly, targeting pathologically enlarged nodes *via* the oesophagus with an endoscope - endoscopic ultrasound - allows sampling of nodes not possible with either EBUS-TBNA or mediastinoscopy.

Coupling diagnostic procedures such as EBUS-TBNA with advanced radiological techniques which help determine areas of disease activity, such as positron emission tomography [124], might improve yields further. Given the rising incidence of drug-resistant disease, these investigations may become an increasingly useful adjunct for the diagnosis of extrapulmonary TB in low-burden countries and may avoid the need for more invasive procedures.

Improving culture of M. tuberculosis *and drug susceptibility testing: microscopic observation drug susceptibility*

The detection and treatment of sputum smear positive pulmonary TB is a key part of the WHO strategy to reduce the global burden of TB; however, ~50% of patients are smear positive at presentation [1]. Traditional culture to confirm disease on solid media can take up to 6 weeks and only after this has been done can the bacillus be tested indirectly for drug susceptibility. The microscopic-observation drug susceptibility (MODS) assay is a novel technique which circumvents some of these problems.

The MODS assay makes use of the fact that *M. tuberculosis* grows faster in liquid media than traditional solid media and also allows direct, simultaneous drug susceptibility testing by culturing the bacillus with first- and second-line antituberculous drugs. In a head to head comparison of MODS, automated *M. tuberculosis* culture and culture on Lowenstein–Jensen medium in patients with and without HIV infection and suspected of TB in Lima (Peru), MODS improved the sensitivity of TB culture to 97.8% from 89% and 84%, respectively, improved time to culture positivity by 6 and 19 days, respectively, and improved time to drug susceptibility results (including testing for multi-drug-resistant *M. tuberculosis*) by 15 and 61 days, respectively [125]. It is relatively low cost and does not require advanced laboratory equipment, making it a potentially important advance for detection of TB in low-income, high-burden countries where the majority of drug-resistant TB is concentrated and has hitherto been poorly diagnosed. The technique does require the training of staff, but even in areas where trained laboratory staff are not available the use of mobile phone technology in the reading of the liquid culture has been promoted as a way to aid roll-out of this promising system [126].

MODS have been assessed in children [127], in patients with HIV infection [128] and in TB meningitis [129]: three situations where improvement in TB diagnosis is essential. In all instances it has been found to be a safe, sensitive and rapid diagnostic tool and in a recent systematic review of the available evidence MINION *et al.* [130] endorse this view.

Improving near patient testing: lipoarabinomannan

One obvious disadvantage of the sputum-based NAAT techniques discussed previously, including Xpert MTB/RIF, is that it still requires patients to be able to expectorate sputum, which is often not possible in young children. Furthermore, there are as yet limited data on its performance in the context

of extrapulmonary disease, where there is the additional complication that invasive techniques may be required to obtain samples for testing [131]. In these situations, a biomarker in blood or urine, which can indicate active TB disease anywhere in the body, would represent a considerable advance. Mycobacterial lipoarabinomannan (LAM), a heat-stable glycolipid, has been suggested as one such biomarker. LAM is specific to mycobacteria and is released by metabolically active bacilli. It is filtered by the kidney and hence detectable in urine. Research so far suggests that it is a specific test for the diagnosis of active TB, but lacks sensitivity, particularly in areas with low HIV prevalence; in areas with high incidences of HIV infection it may be more clinically useful, as the test appears to perform better with increasing immunosuppression [132]. These data have measured urinary LAM using ELISA technology, but dipstick based tests are now also being evaluated which, if successful, would be of potentially significant utility in low-resource settings.

Thus, improving the early diagnosis of active TB and accurately diagnosing drug resistance across the globe is arguably the most pressing concern for TB control at present. While some progress has been made in terms of improving the sensitivity and specificity of tests to diagnose active TB, practical barriers such as provision of infrastructure and training of staff remain significant hurdles in low-income countries. As discussed previously, the Global Plan to Stop TB recognises these problems and is committed to developing solutions to counter them.

UPDATE ON HIV AND TB

While a comprehensive review of TB and HIV is beyond the scope of this article (and has been reviewed elsewhere [133, 134]), the past decade has seen a number of steps forward in our understanding of the interaction of TB and HIV.

In well-resourced settings, patients with TB and HIV co-infection have become an increasingly common sight reflecting the twin epidemics still raging in sub-Saharan Africa. TB incidence has continued to rise in areas worse affected by HIV. One of the major achievements of the past decade has been the great increase in access to antiretrovirals. While this has yet to be clearly reflected in falling TB incidence at national or regional levels, it has allowed more detailed understanding of the impact of highly active antiretroviral therapy (HAART) on TB. For reasons that are not well understood, an individual's risk of TB increases from the first year that they are infected with HIV [135] and the introduction of HAART reduces the risk of TB at all stages of immunosuppression (although most markedly at lower CD4 counts) [136].

Diagnosing TB in individuals with HIV can be more difficult, most importantly because of varied chest radiograph appearances, a lower rate of positivity on conventional smear microscopy and a greater proportion of extrapulmonary disease [137]. In individuals commencing HAART in settings with limited diagnostics, undiagnosed TB probably makes an important contribution to the high mortality seen after initiation of HAART [138, 139]. As outlined previously, the novel Xpert MTB/RIF platform appears able to significantly improve diagnosis of both smear positive and negative disease; if similar performance is achievable in routine practice, management of co-infection could improve significantly, at least where such technology is able to be implemented.

As discussed previously, the performance of urinary LAM assays in HIV-positive individuals has been better than in HIV-negative patients, supporting the hypothesis that immunosuppressed individuals have a greater mycobacterial burden. Such an increased burden is probably a risk factor for immune reconstitution inflammatory syndrome (IRIS) (be it paradoxical in those on TB treatment or unmasking in those who are not [140]). While IRIS remains a fascinating insight into disease pathogenesis and a relatively common challenge to clinical practice, experience has made it a less feared (though still potentially fatal) consequence of co-infection [141]. High-quality evidence is emerging of the benefits of steroids in management but there is still scope for better and more rational therapies to be introduced [142].

The risk of IRIS is one reason why the timing of antiretroviral therapy in relation to TB therapy has been a major source of concern. Data from trials randomising the timing of HAART in relation to TB treatment are beginning to emerge. The first, SAPIT 003, showed that sequential therapy (HAART after TB treatment completion) led to a greater mortality rate than integrated therapy (HAART during first 2 months of TB treatment) [143]. However, many felt that the deaths in this trial were predictable and avoidable and that the more important question was whether HAART should be introduced very early (around 2 weeks) or early (around 8 weeks) [144]. The CAMbodian Early *versus* Late Initiation of Antiretrovirals (CAMELIA) trial in pulmonary TB suggests the former has long-term benefits [145]. This might not be true for TB meningitis where toxicity was greater with early introduction (although the high prevalence of hepatitis C virus in this study might not make it widely applicable to other settings) [141].

Drug interactions between TB and HIV medication are now better understood with a better understanding of antiretroviral drug metabolism induced by rifampicin [146]. Standard dosing of non-nucleoside reverse transcriptase inhibitors appears to be associated with good outcomes for efavirenz and marginally less good for nevirapine where drug interaction may be more important [147]. An increasing challenge will be the treatment of TB in the setting of second-line HIV treatment which usually contain boosted protease inhibitors and therefore cannot be prescribed with rifampicin. Studies are beginning to explore the best dosing of alternative agents (*e.g.* rifabutin) and future work will need to investigate these treatments in large clinical trials [148].

Finally, in parts of sub-Saharan Africa, HIV and TB have combined to fuel a sub-epidemic of MDR- and extensively drug-resistant (XDR)-TB. An outbreak in South Africa was particularly widely covered [149] and cases of XDR-TB have now been identified throughout sub-Saharan Africa and in many other parts of the world [1, 150]. The additional burden of MDR-/XDR-TB carries a high mortality and such cases appear particularly common in healthcare workers [151]. With international worker migration ever more common, such cases are likely to provide challenges in well-resourced settings.

It has to be hoped that sustained resources for HIV treatment and a trend to earlier initiation of HAART over the next decade

will see TB/HIV becoming a less common problem in clinics, but the gap between what could be achieved, and what will be, remains considerable.

UPDATE OF THERAPEUTICS AND MANAGEMENT IN TB

The development of increasingly drug-resistant TB was not matched by advances in TB therapeutics in the past 25 yrs, until very recently. Since the introduction of rifampicin in 1963, no new drugs have reached the hands of clinicians having been specifically developed for use against *M. tuberculosis*, and only one drug, moxifloxacin, has had phase III trials for its use during TB treatment as a first-line agent. Despite this inertia, there has recently been an increased will from governments, academia and non-governmental organisations to develop new drugs for TB and these efforts will be reviewed below.

Ultimately, a vaccine which is completely protective against TB disease, or even TB infection, remains the Holy Grail for many researchers. The current 90-yr-old vaccine, BCG, seems only able to usefully protect against leprosy and protect children from disseminated and meningeal forms of TB (although very recent data suggest it might have a wider effect than this [152]) and as such is sub-optimal. There is, therefore, considerable international effort to develop an improved vaccine for TB; this area is well covered elsewhere and is beyond the scope of this review [153].

Novel drugs to treat TB

The last completely novel first line antituberculous drug, rifampicin, was introduced in 1963. It is only now >40 yrs later and after the appearance of MDR- and XDR-TB that we are starting to see the results of research labour and phase II and III trials for new drugs.

The challenge is not only to find new drugs to treat increasingly drug-resistant TB, but also to find drugs which may allow shorter treatment courses, given the prolonged and occasionally arduous nature of antituberculous treatment for the patient. Fluoroquinolones, such as ciprofloxacin and moxifloxacin, provide one such hope. They have *in vitro* and *in vivo* antituberculous activity and are accepted second-line drugs used in MDR disease. In a phase II, double-blind RCT in Brazil, the substitution of moxifloxacin for ethambutol improved culture conversion at 2 months in a cohort of 170 patients [154]. However, these results were not convincingly replicated in two studies where moxifloxacin was substituted for ethambutol and isoniazid, respectively, where only statistically nonsignificant improvements in 2-month culture conversion were found [155, 156]. A large phase III multicentre trial (REMox TB) is now underway to assess whether moxifloxacin can replace either isoniazid or ethambutol in standard short-course therapy and potentially allow the duration of treatment of pulmonary TB to be shortened. All of these studies necessarily assess the impact of fluoroquinolones on pulmonary TB; it is assumed that the same effect will be found in extrapulmonary disease, although this may of course not be the case.

In addition to the fluoroquinolones, there are six completely novel drugs now in clinical development (SQ-109, PNU-100480, TMC-207, OPC-67683, PA-824 and rifapentine) [157], and two further drugs, linezolid [158] and imipenem [159], which are licensed for the treatment of Gram-positive and negative infections and are used off-license for TB already (table 1). The most promising of these, TMC-207, is a diarylquinoline which inhibits mycobacterial ATP synthase. In a randomised placebo-controlled trial where TMC-207 was added to standard treatment in 47 patients with MDR-TB, addition of the drug significantly reduced time to sputum culture conversion and increased the proportion of patients with culture conversion at 2 months, with minimal side-effects [160]. The second stage of this phase II trial is now underway in a larger cohort of patients. Many more drugs are in preclinical development [157] and it is hoped that at least some of these will reach clinical development and patient testing.

The use of steroids to treat TB and the management of paradoxical reactions in HIV-negative patients

Paradoxical reactions, where TB disease appears to worsen despite ongoing treatment in the absence of drug resistance or drug non-compliance, are a well-recognised phenomenon, particularly in HIV-infected individuals where they are associated with HAART [140].

Widely recognised in association with cervical lymph node disease or central nervous system (CNS) disease in HIV-negative individuals, the underlying immunopathological processes underpinning paradoxical reactions remain unknown, and it can often provide a therapeutic challenge to the treating physician. Although it appears to be a common phenomenon, with small studies estimating its prevalence to be 10–25% of HIV-negative cases, little evidence exists to guide management in these HIV-negative patients, particularly for lymph node TB (where the treatment options are glucocorticoids and surgery), and only one retrospective analysis has been performed to specifically address this problem [161, 162]. This study showed no association between use of steroids and length of reaction; prospective cohort studies assessing both the underlying aetiology of paradoxical reactions and its treatment would, therefore, be of value.

Although much of the morbidity in TB disease results from an excessive cellular host response to the bacillus, the use of steroids generally in the management of TB remains controversial. The earliest positive evidence for their use was in pericardial TB and this indication is now generally supported by national guidelines and a Cochrane Collaboration review [82, 163–166]. While the systematic evidence for their use in TB

TABLE 1 Drugs in clinical development

Class of drug	Name of compound	Stage of development
Fluoroquinolones	Moxifloxacin	Phase III
Diarylquinolones	TMC-207	Phase II
Nitroimidazoles	PA-824	Phase II
	OPC-67683	Phase II
Oxazolidinones	Linezolid	Phase II
	PNU-100480	Phase I
Rifamycins	Rifapentine	Phase I–II
Ethylenediamines	SQ-109	Phase I
Carbapenems	Imipenem	Phase I

of the CNS was originally inconclusive, strong evidence from Vietnam for their use in TB meningitis in adults has been followed by a more recent Cochrane Collaboration review which now supports their use in HIV-negative patients with TB meningitis to reduce the risk of death or disabling neurological deficit [167, 168]. A typical regimen is treatment with dexamethasone 0.4 $mg \cdot kg^{-1}$ every 24 h with a reducing course over 6 to 8 weeks.

Outside these narrow indications, many physicians will often use steroids where the burden of infection is high, and hence presumably evokes a substantial host response leading to life-threatening disease. It should be cautioned, however, that no evidence exists for this approach so it must be taken on a case by case basis.

MDR- and XDR-TB: issues and modern management

The past 30 yrs has seen the emergence of MDR-TB, followed by XDR-TB and more recently TB resistant to all drugs, heralding a return to the pre-antibiotic era [149, 169, 170]. MDR-TB is defined by infection with *M. tuberculosis* which is resistant to both rifampicin and isoniazid, and XDR-TB, where the bacillus is additionally resistant to fluoroquinolones and at least one injectable agent (such as amikacin, capreomycin or kanamycin). Resistance to multiple antituberculous drugs has arisen for a number of reasons; however, it can be argued that the principle reasons for this are the indiscriminate or poorly managed use of antibiotics, coupled with a lack of drug susceptibility testing in regions where it is most needed [171].

The scale of the problem is significant, although accurate figures are difficult to obtain given that in the regions in the world where drug-resistant TB is concentrated, drug susceptibility testing is often not available. The WHO estimated that in 2008 there were 440,000 cases of MDR-TB [1, 7]. Globally, the majority of cases of MDR-TB are found in India and China; in Europe, the majority of cases are in Eastern Europe and former Soviet states. Again, the prevalence of XDR-TB is hard to accurately assess, although it has now been found in 58 countries, representing all geographic regions of the world [1].

How then to manage MDR- or XDR-TB? As noted previously, the regions which are most afflicted are those economically and practically least well equipped to diagnose drug resistance and manage it. Despite this, there have been attempts to critically study the best approaches to management of MDR-TB: whilst most are small, JOHNSTON *et al.* [172] systematically reviewed these data until the end of 2008. They selected 36 articles for final analysis and were able to assess end-of-treatment outcomes for 4,959 patients. 62% had a successful outcome; factors associated with this were the use of surgery and fluoroquinolone sensitivity. Factors associated with a worse outcome included male sex, smear positivity at outset, alcohol use and a low body mass index. Similarly, ORENSTEIN *et al.* [173] found in a meta-analysis of existing data that treatment durations of at least 18 months and those with directly observed regimens were significantly more successful than those without. Individualised regimens were also more successful, although this difference was not significant. National guidelines and a recent expert review agree that the specific drug regimen should always include at least four drugs to which the isolate is susceptible and a single drug should never be added to a failing regimen to minimise resistance [174]. Within this, each drug should be added in a step-wise fashion according to its effectiveness and evidence: a typical MDR regimen for an isolate resistant to rifampicin and isoniazid would therefore be ethambutol, pyrazinamide, a fluoroquinolone such as levofloxacin and an injectable agent such as capreomycin (table 2). The evidence base for these recommendations is limited and far more work is needed if outcomes are to be maximised and side-effects minimised, particularly as new drugs become available.

The first report of outcomes in XDR-TB were from an outbreak in Tugela Ferry, Kwa-Zulu-Natal, South Africa in 2006 [149]. The astonishingly high mortality rate here, where the median length of survival of these HIV co-infected patients was only 16 days, led to concerns that XDR-TB would turn out to be almost untreatable: a throwback to the pre-antibiotic era. XDR-TB cases in Western Europe were also found to have a significantly higher rate of death and hospitalisation time compared with MDR-TB cases [175].

However, a key report from Peru has since shown treatment success in patients with XDR-TB can be similar to those with MDR-TB, where a focussed, individualised programme was used [176]. SOTGIU *et al.* [177] reviewed the available literature on XDR-TB, finding the data collected too methodologically

TABLE 2 Pharmacological management of multi-drug resistant (MDR) tuberculosis (TB)

First-line oral drugs	Injectable bactericidal drugs#	Fluoroquinolones#	Second-line bacteriostatic drugs	Other drugs with unclear or unproven efficacy
Rifampicin¶	Streptomycin	Moxifloxacin	Cycloserine	Clofazimine
Isoniazid¶	Amikacin	Gatifloxacin	*p*-aminosalicyclic acid	Clarithromycin
Rifabutin	Kanamycin	Ofloxacin	Prothionamide	Amoxicillin/clavulanate
Ethanmbutol	Capreomycin	Levofloxacin	Ethionamide	Linezolid
Pyrizinamide			Terizidone	Imipenem/cilastatin
				Thioacetazone
				High-dose isoniazid
Order in which drugs are added to MDR-TB regimen →				

#: all MDR-TB regimens should include one injectable and one fluoroquinolone where possible; ¶: by definition, isolates classed as MDR will be resistant to these drugs.

heterogeneous to form a meta-analysis to determine outcome predictors. From their systematic review, they have recommended a number of areas where improved data collection strategies might shed more light on how to achieve better outcomes in XDR-TB, and agree that treatment outcomes appear significantly better than first described in 2006. Similarly, in reviewing the small amount of data published on treatment outcomes in XDR-TB, JACOBSON *et al.* [178] found ~44% of patients to have a successful outcome, and that fluoroquinolone use (even where one would expect it not to be helpful) was surprisingly associated with this. MIGLIORI *et al.* [179] support the key importance of fluoroquinolone sensitivity in outcomes from drug-resistant TB, where fluoroquinolone-resistant MDR-TB cases in Italy, Germany and Estonia had a higher proportion of treatment failures or deaths compared with fluoroquinolone-sensitive cases.

CONCLUDING REMARKS: A NEW DECADE

Whether these outcomes in drug-resistant disease, which have all been in the context of well-organised treatment programmes, are possible in day-to-day practice remains to be seen. However, these data show it is possible to succeed in situations where therapeutic nihilism is the only other alternative.

Similarly, there have been major advances over the past decade in our understanding of TB epidemiology and pathogenesis, which have started to lead to the development of novel tests and therapies for the disease. Much of this has been a direct consequence of the significant investment in TB research and management by academia, governments, charitable foundations and non-governmental organisations over the past 5–10 yrs, directed by the WHO's Stop TB Strategy [10].

However, as we enter a new decade where the continued challenge to find better diagnostics and therapeutics for the management of TB occurs in a world confronted by increasingly drug-resistant isolates and interconnected geopolitical and economic crises, we would do well to remember that it will not just be political will that determines successful outcomes, but the will and commitment of individual physicians and their patients. As William Osler said of TB, prior to the use of chemotherapy: ''A rigid regimen, a life of rules and regulations, a dominant will on the part of the doctor, willing obedience on the part of the patient and friends – these…are necessary in the treatment of pulmonary tuberculosis'' [180].

STATEMENT OF INTEREST

M. Berry and O.M. Kon are named co-inventors on a patent application for the use of transcriptional signatures as biomarkers in tuberculosis, international application no. PCT/US2009/048698.

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