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TB Summit 2014

Prevention, diagnosis, and treatment of tuberculosis—a meeting report of a Euroscicon conference

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†Dr Bhakta was the chairperson on March 26, "TB Summit 2014: Treatment"

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World TB Day commemorates Dr Robert Koch's first announcement on March 24, 1882, that the bacterium *Mycobacterium tuberculosis* is the causative agent of tuberculosis. Currently, the event comprises of several conferences, meetings and activities held all over the world with the singular intention of raising public awareness about the global health emergency.

In spite of having discovered the etiological agent of tuberculosis more than a century ago, a sizeable population still contract the disease every year and fall prey to it. In 2012, an estimated 8.6 million people developed the disease with 1.3 million succumbing to it. The number of TB deaths in children is unacceptably large, given that most are preventable. However, the challenge appears to be shifting toward attempts to control the rise and spread of the drug resistant variants of the microbe. To achieve this, a concerted effort from academia, clinical practice, and industry has been put forth.

The TB Summit 2014 attempted to raise awareness as well as bring together experts involved in different aspects of tuberculosis research to help establish a more collective approach to battle this age-old disease.

Introduction

After last year's success at organizing a meeting to commemorate World Tuberculosis (TB) Day,[1] Euroscicon expanded its scope to include in-depth discussions on the latest advancements in the prevention, diagnosis and treatment of TB, over a three-day long (24th March – 26th March) international conference. It was a well-attended conference comprising of about 30 oral and poster presentations of primary data and a few lectures, hosted by a renowned set of clinicians, practitioners, scientists and industry executives, all involved in the day-to-day fight against TB. The O_2 Arena in East London served a fitting venue for the event, in light of the recent finding that London had the highest incidences of the infectious disease in all of western Europe.[2]

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Prevention

Dr Derek Sloan (Liverpool School of Tropical Medicine, UK), was the chairperson for the discussions of the day, which primarily focused on prevention of transmission of TB as a means of disease control. TB has been with mankind for millennia and still affects the lives of a significant portion of the world's population. The World Health Organisation (WHO) and Stop TB partnerships have laid down ambitious goals aiming toward eliminating TB as a global health problem by 2050, however, many regions are already failing to achieve the set milestones.[3] The association of the disease with poverty was elucidated by Dr Sloan to set the tone for the day's speakers. It is a disease of the vulnerable masses, living in cramped situations with no access to a public health system. His view is further validated by the rise of TB incidence in Eastern Europe that occurred in pace with the breakdown of the Soviet Union and its public health system.[4] Finally, the HIV co-epidemic has inflicted the final blow on existing high-burden, resource-poor countries. Attempts to control such a contagious disease primarily focused on prevention of *M. tuberculosis* infection by preventative vaccines, chemo-prophylaxis or restricting transmission of the TB-pathogen by maintaining the standard health and hygiene standards at home or in a community as well as early detection and drug treatment.

Stressing the need to detect patients early on so as to prevent transmission of the disease to their close contacts was the main theme of the lecture by Professor Juraj Ivanyi (King's College London, UK).[5] The current guidelines on detection miss cases until the disease has progressed and been transmitted. This shortcoming along with protracted chemotherapy and the absence of an effective preventative vaccine are the primary reasons why TB exists in the developed world which offers higher living standards. However, detecting patients sooner would require a diagnostic test with sensitivity and specificity far higher than those achievable with methods currently in use. A firm proponent of serological tests for the diagnosis of TB,[6] he mentioned the need for further research to identify specific antigens that

induce antibody formation in the serum of TB patients found to be smear negative. The immediate advantage of these tests over the conventional methods of microscopy and smear culture tests would obviate the arduous task of sputum collection while increasing the sensitivity and specificity of diagnosis. He reiterated his view that serological tests in combination with an organized system of early detection, by making changes in the guidelines for recording "mild symptoms" and active case-finding in communities with high incidence should receive as much emphasis as development of novel drugs, drug regimens and their treatment outcomes. While Professor Ivanyi discussed a list of antigens that could be used as a kit for prospective clinical trials, the effectiveness of a test relying on an immune response from patients who, in most cases, are immunocompromised remains to be seen.

Detection of latent TB infections (LTBI) followed by prophylactic treatment is also viewed as a probable strategy to prevent activation of the disease followed by its transmission to new patients. QuantiFERON®–TB Gold, an interferon gamma release assay (IGRA) provided by Qiagen was one of the FDA approved diagnostic tools discussed in the conference. Chris Wilson (Qiagen Ltd.) showcased the improvements made in the latest kit, which includes three tubes—TB-antigen tube (containing an antigen TB7.7 in addition to the previously used ESAT-6 and CFP-10), mitogen tube (to detect non-specific interferon gamma release) and a negative control (no antigen) tube (http://www.qiagen.com). Increasing the number of antigens used and laying down precise indeterminant reading criteria make the test reproducible and specific with fewer indeterminant results. The expense of the test is still prohibitive for use in resource-poor countries; however, Synlab's (http://synlab.co.uk) outbreak service works in collaboration with Qiagen to detect TB accurately and control devastating outbreaks.

In addition to this, Dr Philip Monk (Public Health England, UK) emphasized the use of genomics to help trace transmission events and improve TB surveillance. Genotyping TB isolates from patients enables the confirmation of epidemiologically linked cases, detection of lab cross contamination, and differentiation between reinfection and reactivation of disease. MIRU/VNTR[7] has gained a lot of success in being able to detect cases early and enable rapid initiation of treatment; however, it cannot differentiate between primary, secondary, or tertiary infection, hence the source remains unknown. With the advent of whole genome sequencing (WGS), it is now possible to obtain a better resolution of strains studied and understand a transmission event. In spite of the genetic stability of *M. tuberculosis*, WGS can differentiate between strains and indicate the direction of the transmission. WGS can identify time to the most recent ancestor, thus rapidly detecting drug resistance, which can lead to immediate therapeutic intervention. It has the potential to simplify workflow, reduce turnaround time, and can be a game-changer if practiced in conjunction with tighter guidelines for contact tracing. The need to prioritize contact tracing was the main theme of the best poster award-winner of the following day, presented by Mr S.J. Patel (Aberhart Centre, Canada) who discussed TB transmission in the aboriginal population of Alberta and Saskatchewan.

Moving on to prevention based on host immune responses and vaccines, Professor Tom Ottenhoff (Leiden University Medical Centre, Netherlands) discussed the possible reasons for the disappointing results of clinical trials of new vaccine candidates, along with laying down hypotheses to develop more effective ones in the future.

As $CD4^+$ Th cells are strongly associated with protection against TB, a vaccine that induces a strong response from these cells qualifies as a promising, potential vaccine candidate. However, T-cell response is measured using an interferon assay, which might not be a suitable biomarker to gauge the response of the various T-cell populations. The case of MVA85A,[8] a new vaccine candidate that showed an impressive immune profile in all animal models and yet exhibited no higher protective efficacy over BCG vaccines in human trials,[9] illustrates the importance of the antigens selected for vaccine development. Professor Ottenhoff added that the protective efficacy of MVA85A might have been masked by BCG and that it may perform better against the more severe forms of TB. Focusing on antigen selection, Professor Ottenhoff emphasized learning from the microbe, his main hypotheses being that the best vaccine candidates would arise from antigens that are expressed either during active/latent infection or at the primary site of infection, the lungs. Very little is known about the antigen repertoire of *M. tuberculosis*. Only a fraction of the genome has been mined for antigens[10] and there is little knowledge of the vaccine potential of most antigens. In the initial phase of the infection the bacilli secrete early secretory proteins; however, on progression of the infection, under hypoxic conditions, the bacilli undergo dramatic changes in their expression profile to resist the environmental stress. These include the upregulation of a set of approximately 48 genes that belong to the dormancy survival regulon or the dosR regulon.[11] Hence, these proteins along with other starvation proteins could be more useful in inducing protective immunity,[12] rather than the BCG vaccine, as *M. bovis* BCG does not enter a phase of latency like its infectious counterpart. In vivo expressed (IVE) TB antigens that induce strong $CD4^+$ and $CD8^+$ T-cell responses in peripheral blood mononuclear cells (PBMC) in long-term latently infected patients[13] have also attracted interest as vaccine candidates owing to promising results seen with primed T-cells in macrophage infection models. These antigens could offer future vaccine candidates. The main blockades to vaccine development, however, remain the lack of predictive animal models and effective biomarkers in the human context.

Diagnosis

Professor Philip Hill (Centre for International Health, New Zealand) introduced the main challenges of TB control and management on the second day of the conference, which focused on diagnostics. With more than a third of the world's population already infected with the TB germ, vaccines and passive case detection are far from being the central players in the control of TB. TB being a biphasic infection, active case-finding along with prophylactic treatment should ideally avert a large number

of reactivation cases as seen previously.[14] However this is not the case as current diagnostic systems do not have the required sensitivity to detect all the TB-positive cases while being specific enough to exclude all the TB-negative individuals from needless therapy. To study the possibility of eliminating TB by prophylactic treatment alone, the geographically isolated island Republic of Kiribati with an annual incidence of 350 new cases per 100 000 has been selected. The rationale behind the study is to offer a short course anti-TB treatment to all on the island so as to clear active and latent TB cases while protecting the uninfected individuals. Modeling exercises revealed that controlling the relapse rate is essential for successful control of the disease and accurate diagnosis is vital to avoid relapses. Diagnostic tests that can accurately narrow down the number of individuals that should be given prophylactic treatment are a necessary requirement for the elimination of TB. The tuberculin skin test (TST), in addition to being cheap, has been shown to give better results than the other commercial tests based on IGRA currently available, in studies performed in Gambia.[15,16] Professor Hill emphasized the need to identify new targets for the development of more effective diagnostic tests, and suggested investigating the biomarker profiles of early clearance initiated by the innate as well as adaptive immunity.

A returnee speaker from last year, Dr Richard M. Anthony (KIT Biomedical Research, Netherlands) gave an update of their studies on the "treat to test" strategy.[17] Modifying the original idea to a pragmatic approach to look at patient response to therapy, they have devised the MOTET, or "Monitoring of the effect of tuberculosis treatment". They suggest that vital information is lost due to the lack of collection of biomaterial in the initial phase of the treatment. An initial burst of bactericidal action on the start of treatment is observed in infected individuals and could be exploited as a diagnostic tool with potential to detect even multidrug-resistant (MDR) strains. This response along with non-specific bactericidal action, cytokine levels and drug toxicity indicators could provide useful information that needs to be carefully studied. Placebo controlled, infection controlled experiments in mouse models showed interferon gamma and IP10 as effective indicators of infection as these do not show up in non-infected mice. A trial monitoring cytokine levels in human subjects in Nepal indicated that a fraction of smear negative patients could actually be non-infected individuals receiving unnecessary treatment; however, the need for a larger trial to provide definitive answers was expressed.

Diagnosis of TB in children is a much debated topic. A majority of the deaths in children can be attributed to TB, most of which are drug-susceptible and can be easily treated, but are routinely missed due to difficulties in diagnosis. Microscopy and culture are not good tests to detect TB in children as collection of sputum is difficult. In addition, there is a high level of discordance between IGRA and TST results obtained from the limited pediatric data available. Dr Marc Tebruegge (University of Southampton, UK) discussed attempts to reveal the underlying mechanism of discordance and mentioned that the discordants are a heterogeneous group with previous BCG exposure not being an explanation for the responses to the tests being performed.

Tuberculosis is a zoonotic disease, easily transferred from animal to man and is a crisis only recently being looked at. The more unorthodox reservoirs of pathogenic mycobacteria, such as cats and camelids, were discussed by Professors Danielle GunnMoore (University of Edinburgh, Scotland) and Shelley Rhodes (Animal Health and Veterinary Laboratories Agency, UK), along with the diagnostic tools implemented to detect TB in these animals. Cats are inherently resistant to *Mycobacterium tuberculosis*, but do acquire *M. microti* and *M. bovis* infections.[18,19] Infections are mostly cutaneous in nature, manifesting as isolated lump/s. For *M. microti*, cats are a dead-end host as it does not spread even among them. *M. bovis* however, is spread easily through bleeding skin lesions that contain multi-bacillary *M. bovis*. Picked up from rats, the *M. bovis* bacilli can spread rapidly, as seen in a cluster of cats studied in Newbury. Progression of disease in cats is much faster than expected rates, and passage through an unnatural host was offered as an explanation for this phenomenon. In light of the rapid transmission and progress of TB in cats, an effective diagnostic tool is imperative. No serological data has been established; however, IGRAs give dependable results, as do radiographs to some extent.[20] Conventional techniques of culture and PCR serve well but are restrictive either due to time or monetary constraints.

Discussing the problems of TB surveillance for alpaca herds, Dr Shelley Rhodes revealed that half of the alpaca herds in the UK are in areas endemic to bovine TB. Alpacas and other camelids are susceptible to TB, and being highly social animals the disease gets transmitted within a herd fairly quickly and alarmingly to the owners as well. These animals show little or no consistent symptom of TB and by the time infected animals are identified their pathophysiology is severe. Diagnosis using TST is not ideal as its sensitivity is very low in alpaca herds, with a higher likelihood of missing an infected animal than detecting it. Recent studies found IGRA to perform better than serological tests at TB detection,[21] though there are plans for testing the use of a combination of antibodies to detect TB. PCR-based tests have also been shown to have the highest specificity of all at the cost of the sensitivity of the test.

An issue plaguing the conventional forms of smear culture diagnosis is the time required. Dr Catherine E.D. Rees (University of Nottingham, UK) discussed the use of bacteriophage-based FAST-Plaque assay, which exploits the selectivity and rapid doubling time of bacteriophages to develop a rapid, specific method that detects any slow growing mycobacteria, including human and bovine TB within 48 h. The test, already successful in TB detection,[22] has also been useful in the detection of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) that causes Johne disease. The test picks up bacilli from blood samples of infected cattle before any clinical signs of disease, and also from ELISA-negative animals. A new high-throughput assay format and the possibility of the test serving as a differentiating infected from vaccinated (DIVA) test for bovine TB is also under investigation. The FAST-Plaque assay is performed by incubating the target pathogenic mycobacterium-containing sample (blood, sputum, milk) with bacteriophage, thereby loading the cells with the virus particles. A virocide is then used to kill any particles

that have not infected cells. The solution is overlaid on a lawn of non-pathogenic mycobacteria. The viruses replicate within the mycobacteria and visible plaques are formed. This test, unlike the molecular detection techniques detects the presence of only viable cells and is much faster to complete, taking 48 h instead of the 14 d required to culture pathogenic mycobacteria. Preservation of DNA in the plaques formed on agar plates is a beneficial outcome as it allows for strain typing as well.

One of the best posters of the event also discussed the problems of the conventional methods of bovine TB detections. Dr L.D. Stewart (Queen's University, Northern Ireland) described a novel lateral flow device that uses binders produced to pathogenic *M. bovis* and is specific to it and can easily differentiate between *M. tuberculosis* and *M. bovis* infections.

Treatment

Discussions about diagnostics invariably led to the common theme of drug treatment and the rapidly diminishing family of drugs that are still effective to treat drug-resistant forms of TB. While treatment regimens for drug-susceptible TB in humans have been validated and in use for several years, the 6-mo course is too lengthy and needs to be reduced if patient compliance issues are to be tackled. The primary reason behind the lengthy treatment regimen adopted for treating TB is the occurrence of bacillary sub populations that differ in replication rates, metabolism, and drug sensitivity and get cleared from the body at different time points, thus giving rise to a biphasic bacterial elimination pattern.[23] Tackling resistance and reducing treatment regimens will require identification of lead molecules with anti-tubercular property that can be modified and developed further as drugs for tuberculosis chemotherapy. However, gold-standard high-throughput methods of identification of novel anti-tubercular agents using appropriate surrogates also need to be developed. One such method, the spot culture growth inhibition assay (SPOTi) was demonstrated through a short film launched by the Mycobacteria Research Laboratory (Birkbeck, University of London, UK) at the conference.[24-27]

A number of essential enzymes involved in key metabolic pathways are being investigated to identify their potential as likely targets for the development of anti-tubercular drugs with novel mechanisms of action that can curb the rise of the drug-resistance in the organism. The cell wall has long been the target of choice when attacking bacteria; however, the peptidoglycan (PG) layer and its metabolism might still offer new enzymes such as the ATP-dependent Mur ligases[28,29] and other key players involved in the degradation, transport and recycling of the macromolecule (poster presented by Ms F. Scotti, UCL School of Pharmacy, UK). Professor Edith Sim (Kingston and Oxford University) has an established interest in identifying targets essential for intracellular survival of the pathogen. Mechanisms enabling the bacteria to survive intracellular conditions, though not necessarily involved in persisting, are still of paramount importance. One such mechanism is cholesterol metabolism. Cholesterol is a potential fuel for the organism once inside the macrophage.[30] The enzyme HsaD is involved in dismantling the steroidal nucleus of cholesterol, thus providing energy for sustenance of the cell.[31] A two-way approach of studying the effects of genetic modification and chemical inhibition of enzyme activity detected using a high-throughput colorimetric assay[31] was performed. The knockout mutant failed to grow in the presence of cholesterol as expected. The high-resolution crystal structure of HsaD in its apo- and ligand-bound form enabled the identification of its active site, which fortunately is ideal for fragment-based drug discovery. This strategy relies on increasing affinity of the ligand molecule to the enzyme by linking low affinity ligands together. Assays for binding and inhibition were used and a sulphonamide that inhibits the activity of HsaD has been identified.

Another gene from the operon that *hsaD* belongs to is the *nat* gene, which encodes for the protein arylamine *N*-acetyl transferase (NAT).[32,33] NAT was initially characterized in humans and is present in two isoforms (fast acetylator and slow acetylator). It is implicated in the metabolism of the common anti-tuberculosis drug, isoniazid. A similar approach as with HsaD was used in the case of NAT. Knockout mutant strains showed differences in growth, morphology, mycolic acid synthesis, and an increased susceptibility to drugs and intracellular killing mechanisms.[34] A rapid method to assay enzyme activity enabled them to screen a unique library of 500 compounds out of which two chemical entities were discussed.[35] The compounds themselves though not toxic to the cells in vitro, inhibit growth of the bacteria within the host macrophage cells. Piperidinols, specifically, are more likely to bind covalently to the active site thereby blocking any enzymatic activity.[36] Investigation of the unique *nat* operon in fast-growing mycobacterial species led to the identification of a novel oxidoreductase from *M. smegmatis* which was initially mis-annotated in genomic databases as a dihydrofolate reductase along with a transcriptional regulator *araC*.[37]

Characterization of enzymes involved in dormancy, especially those regulated by the DosR regulon could lead to the identification of novel therapeutic targets. Inhibition of flavin-dependent enzymes in dormancy was demonstrated by a functional assay involving Acg protein[38] (highly upregulated in dormancy) and an oxygen-insensitive nitro-reductase was discussed through a poster by Ms Doris Quay (Birkbeck, University of London, UK).

Natural products are also considered to be the most likely source[39-42] from which to develop novel anti-tubercular agents, as was discussed through poster presentations by Ms C.A. Danquah (UCL School of Pharmacy, UK) and Ms J. Zhao (University of Strathclyde, UK). However, a molecule requires three decades of development and trials before it can reach the market. A strategy used to circumvent the time and investment required is repurposing already available, non-toxic drugs and repositioning them as anti-tubercular drugs. The potential of repurposing non-steroidal anti-inflammatory drugs (NSAIDs) as anti-tubercular therapy was discussed by Ms A. Maitra (Birkbeck, University of London, UK).[24,43,44]

Promisingly, a number of novel drugs and treatment regimens are in various phases of clinical trials at present and bedaquiline, the first FDA-approved anti-tuberculosis drug in 40 y, offers a lot of hope in treating MDR-TB implications.[45-47] However,

clinical trials meant to evaluate the efficacy and safety of novel chemical entities are also in need of investigation and development. Dr Derek Sloan discussed the limitations of phase 2 studies that routinely consider culture conversion after two months of the start of therapy as the end-point. This binary endpoint could be misleading and he suggested the use of bacillary load reduction as an alternative. In a sputum culture study performed in 169 patients they used both solid media as well as MGIT to detect bacillary elimination and developed models based on the data obtained. Solid culture studies showed biphasic pattern of bacillary elimination where a steeper sterilization curve correlated with patients who did not have a relapse. The results of the MGIT system fit a linear model wherein a steeper slope again represented a favorable clinical outcome. Detection of cells with lipid bodies (as discussed by Professor Mike Barer in the meeting last year) in sputum did not affect clinical outcome however patients who failed therapy persistently had higher lipid body positive cells at 2 mo from the start of therapy. Comparing individual plasma levels of drug concentrations also revealed that if plasma exposure to isoniazid was higher in the sterilization phase the bacillary elimination models were steeper.

One of the ten best-selling drugs in the world, adalimumab, used to treat rheumatoid arthritis (RA) increases the risk of patients contracting TB, bringing complications for RA treatment. Dr Tomoshige Matsumoto (Osaka Anti-Tuberculosis Association, Osaka Hospital, Japan) discussed the dynamics of anti-rheumatoid treatment using biologics and anti-TB therapy. Current practices suggest withdrawal of anti-tumor necrosis factor (TNF) treatment as soon as TB is detected and to start it only 2 mo after starting TB therapy. Studying the effects of abrupt cessation of the anti-TNF therapy has showed that it might be more detrimental for the patients, and he suggests continuing the anti-TNF treatment with occasionally a lower dose alongside the regular anti-tubercular treatment.[48]

Treatment of non-pulmonary forms of TB is often overlooked and could lead to a crisis of the different kind. Urogenital TB (UGTB), the most common form of the disease after pulmonary TB, is responsible for both male and female infertility, as pointed out by Dr Ekaterina Kulchanvenya (Novosibirsk Research TB Institute, Medical University, The Russian Federation).[49-51] Usually mistaken for other urinary tract infections (UTIs), patients receive non-effective treatment leading to the positive selection of MDR strains as well as complications arising out of *E. coli* co-infection of the tissues damaged by the TB bacilli. Live and infectious TB bacilli have been isolated from epididymis, prostate, and the urinary tract, thus allowing the risk of non-conventional means of transmission of the disease. Low awareness of UGTB among practitioners leads to delayed diagnosis, which complicates the disease. Chemotherapy for UGTB is not optimized; effectiveness of drugs and their dosage levels as well as the routes of administration need to be investigated to develop a targeted treatment regimen for UGTB.[52] Intravenous administration of drugs is supported by Dr Kulchanvenya as it helps the full dose reach, the plasma avoiding the negative effects of the drugs on the gastrointestinal tract and protecting the patient from the effects of drug toxicity. A main theme of her talk was to raise awareness about the lesser known forms of TB as well as underline the need for early diagnosis.

Carolyn Tauro (Tata Institute of Social Sciences, India) shared results of a survey that collected information on practices of TB control in children in India. A failing public healthcare system in the country necessitates the need for patients to resort to private practitioners. A large proportion of these practitioners consider X-ray results only, thereby undermining sputum smear findings. They do not consider contact history and about half of those surveyed do not advise prophylactic treatment. Though a web-based notification system made mandatory for MDR indications has been set up by the Indian government, the lack of any strong regulatory body governing the practices of the private healthcare industry has made monitoring and ensuring appropriate practices are performed largely impossible. These failings in TB surveillance and control need to be overcome for the new diagnostics and drugs to be successful at beating the disease.

Conclusion

The event could not have been a success without the active participation of all invited speakers, panelists, poster presenters and the audience. The question-and-answer sessions were intensive, intellectually stimulating and probably laid down seeds for further research and investigation via inter-disciplinary collaborations. Meetings such as these are necessary and paramount for the scientific community to exchange technical know-how and work on collaborations to accelerate the process of eliminating TB from the world.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Euroscicon organized this event (www.lifescienceevents.com).

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