7th Annual
TB Research Day

March 22, 2019

Glen • RI-MUHC Auditorium (ES1.1129) & Atrium • 11:30 - 17:30

Interactive Research and Development South Africa
Photographer: Wade Howard

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Program

11:30 - 12:30	Poster set-up, registration and lunch boxes
12:30 - 13:30	Poster session 1 Jean-Yves Dubé (Dr. Behr) 2 Sarah Danchuk (Dr. Behr) 3 Shannon Duffy (Dr. Behr) 4 Sophie Huddart (Dr. Pai) 5 Monica Dallmann-Sauer (Dr. Schurr) 6 Wilian Correa de Macedo (Dr. Schurr) 7 Giorgia Sulis (Dr. Pai) 8 Mikashmi Kohli (Dr. Pai) 9 Paulami Sen (Dr. Pai) 10 Jaryd Sullivan (Dr. Behr) 11 Syed Abidi (Dr. Khan) 12 Jordan Kruisselbrink (Dr. Behr) 13 Eva Kaufmann (Dr. Divangahi)
13:30 - 14:30	Oral presentations (10 mins + 5 mins questions) 1 Nowrin Hoque (Dr. Behr) 2 Jonathon Campbell (Dr. Schwartzman & Dr. Menzies) 3 Diane Singhroy (Dr. Pai) 4 Hannah Alsdurf (Dr. Menzies)
14:30 - 14:45	Year in review and release of Lancet TB Commission Dr. Madhukar Pai
14:45 - 15:00	Launch of the WHO Collaborating Centre for TB Research Dr. Dick Menzies, Dr. Bruce Mazer, Dr. Martha Crago & Dr. Sylvain Baillet
15:00 - 15:05	M[i]4 and TB Centre Dr. Marcel Behr
15:05 - 15:30	Awards and prizes Poster and oral presentations, 2018 publication prizes, 2019 travel awards and TB Centre logo prize
15:30 - 16:00	Coffee and snacks
16:00 - 17:00	Keynote lecture: Dr. Joia Mukherjee Associate Professor, Harvard Medical School Chief Medical Officer, Partners in Health
17:00 - 17:05	Group photo
17:05 - 17:30	Drinks and snacks
17:30 - 20:00	TB film festival

Dr. Joia Mukherjee

Associate Professor, Harvard Medical School
Chief Medical Officer, Partners in Health

TB Care as a Crucial Step Towards Universal Health Care

Biography

Joia Mukherjee, MD, MPH, is an Associate Professor at Harvard Medical School and Chief Medical Officer of Partners In Health (PIH), an international medical charity dedicated to providing a preferential option for the poor in healthcare. She is an internist, a paediatrician, an infectious disease doctor and public health specialist. Dr. Mukherjee has been supporting PIH's efforts to provide high quality, comprehensive health care to the poorest in partnership with local communities and health officials in Haiti, Rwanda, Lesotho, Malawi, Sierra Leone, Liberia, Peru, Mexico, Russia, Kazakhstan and the Navajo Nation. Dr. Mukherjee's clinical foci include HIV, multi-drug resistant tuberculosis, mental health, ebola, human resources for health, and health systems strengthening. She also teaches Global Health Delivery, social medicine, infectious disease and human rights to medical students, residents and fellows at a wide variety of US and international institutions. She has helped create a new residency and fellowship training program for Rwandan and Haitian physicians as well as global health residencies and fellowships for US trainees at Harvard and other American universities.

Poster #1

Mycobacterial molecular patterns work in synergy to complete Freund's adjuvant

Jean-Yves Dubé[1], Fiona McIntosh[2], Nimara Asbah[2], Damien Montamat-Sicotte[2], Juan Zarruk[3], Samuel David[3], Jérôme Nigou[4], Marcel Behr[5]

Jean-Yves Dubé, PhD student

Supervisor: Dr. Marcel Behr

Exposure to mycobacteria normally generates cell-mediated immunity (CMI), such as during natural infection with Mycobacterium tuberculosis (Mtb), and animal immunization with complete Freund's adjuvant (CFA, killed Mtb in oil). The pathogen-associated molecular patterns (PAMPs) of mycobacteria responsible for adjuvancy remain unclear. Cell wall muramyl dipeptide (MDP) and mycomembrane trehalose dimycolate (TDM) have individually been suspected. Mycobacteria distinctly make N-glycolyl MDP, with unique immunological properties, and TDM. We hypothesize that mycobacterial PAMPs work synergistically to elicit CMI, beneficial to Mtb's lifecycle, but inadvertently led to the immunological revolution of CFA-based models of immunization.

We are working to a) demonstrate essential PAMPs of mycobacteria that elicit CMI in vivo by reverse genetics of mice (e.g. knockout of Nod2 and Mincle, sensors of MDP and TDM respectively) and mycobacteria (e.g. knockout of N-glycolylation enzyme namH); b) build a molecular adjuvant from purified or synthetic mycobacterial PAMPs which elicits CMI like CFA; c) demonstrate efficacy of molecular adjuvants in the experimental autoimmune encephalomyelitis (EAE) model.

We show CMI from CFA is both Nod2- and Mincle-dependent. Immune responses similar to CFA were achieved when N-glycolyl MDP was mixed with purified or synthetic TDM. This synthetic formulation produced EAE in mice comparable to the standard CFA. Our research outlines how to replace CFA with a consistent, molecularly defined adjuvant to inform the design of immunotherapeutic agents and vaccines benefitting from CMI.

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Poster #2

Utilizing oligo-mediated recombineering to explore natural variability among BCG strains

Sarah Danchuk[1,2,3], Nowrin Hoque[1,2,3], Marcel Behr[1,2,3,4]

Sarah Danchuk, PhD student

Supervisor: Dr. Marcel Behr

Since the determination of the M. tuberculosis genome and the advent of comparative genomic tools, it has been possible to document genetic variability among BCG strains. While a number of in vitro and in vivo phenotypes have been associated with a particular BCG strain, until now it has been cumbersome to remake each genetic variant, hindering our ability to mechanistically link phenotypes to genetic variants. Our lab aims to use the pNIT:ET recombineering system to engineer mutations, in combination with plasmid-based gene complementation, as a means of studying antibiotic susceptibility as an in vitro phenotype. As previously described, BCG Danish, a common vaccine strain used internationally, has a higher MIC to isoniazid (INH) than other strains, and a mutation in Rv1676c specific to this strain is being evaluated as the potential cause of this phenotype. Further, BCG strains that contain the region of deletion 2 (RD2) have a lower MIC to clofazimine (CFZ), an antibiotic used to treat multi-drug resistant tuberculosis (MDR-TB). Restoration of the Rv1979c gene in BCG Pasteur (RD2-absent) led to a reduction in the MIC similar to that observed with BCG Russia (RD2-present). Interestingly, resistance to both CLZ and bedaquline (BDQ) has also been linked to SNPs in Rv0678. To study this dual resistance phenotype, and separate it mechanistically from CLZ mono-resistance, we have engineered a Rv0678 mutation. Moreover, in ongoing studies, we are using the isogenic BCG mutants generated by recombineering to determine the cause of the antibiotic resistance phenotype, taking advantage of this natural variation. Notably, many of the mutations we have identified, and are studying, are not currently linked to drug-resistance in the clinical laboratory. As such, our findings have the potential to inform the next generation of antibiotic resistance assays and may determine the validity of associated resistance mechanisms in an avirulent strain.

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Poster #3

Investigating the prevalence of zoonotic TB in Vellore, India

Duffy S[1,2,3], Srinivasan S[4], Michael JS[5], Kapur V[4,6], Behr M[1,2,3,7]

Shannon Duffy, MSc student

Supervisor: Dr. Marcel Behr

In 2017 there were over 2.7 million cases and over 400,000 deaths from TB in India alone. The End TB Strategy imposed by the World Health Organization aims to reduce TB-related deaths by 90% by 2030. The Mycobacterium tuberculosis complex (MTC) consists of M. tuberculosis as well as other species including M. bovis and M. orygis. The latter are typically associated with non-human hosts, but can be transmitted to humans, to cause zoonotic TB (zTB). To date, published studies on zTB have been limited in searching for M. bovis, therefore they may have underestimated zTB by not looking for other zoonotic subspecies. If we are to reduce TB related deaths by 90% by 2030, it is imperative that we understand how transmission is occurring and whether strategies focused on human exposures are sufficient. We developed two deletion-based conventional PCR assays to differentiate between M. tb, M. bovis, and M. orygis: a three-primer PCR to detect the region of difference 9 (RD9) and a six-primer PCR to detect differences in the deletion size of RD12. In a pilot study, we analyzed 600 clinical samples (300 pulmonary, 300 extrapulmonary) in Vellore, India to evaluate the prevalence of zTB infection and investigate the potential role of M. orygis. We determined that 19/600 (3.2%) of samples were identified as MTC other than M. tb. Of the 300 extrapulmonary samples, 4 (1.3%) were identified as M. orygis. In this pilot study, we were able to validate the application of our assays to differentiate between MTC species with clinical samples and identify the presence of both M. orygis and M. bovis in human samples, in southern India. Further studies need to be conducted on representative samples from other areas of India, and other high-burden countries, to determine the burden of human TB due to zoonotic infection.

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Poster #4

Case fatality among Indian tuberculosis patients: a systematic review and meta-analysis

Huddart S[1,2], Svadzian A[1,2], Nafade V[1,2], Satyanarayana S[3], Pai M[1,2]

Sophie Huddart, PhD student

Supervisor: Dr. Madhukar Pai

Background

More than a quarter of the global TB deaths occur in India. Patient mortality is an important marker of care quality as prompt diagnosis and appropriate treatment should prevent deaths both during and after treatment. This systematic review seeks to estimate the case fatality ratio (CFR) for Indian TB patients.

Methods

We searched Medline, Embase and Global Health for eligible papers published between 2006 and 2017. The treatment and post-treatment CFRs were extracted and, when sufficiently homogeneous, pooled using Normal-Binomial Generalized Linear Mixed Models. Pooling was also performed in key patient subgroups.

Results

A total of 125 relevant studies were identified. The overall treatment CFR was 0.06 (95% CI: 0.04, 0.07). The CFR was higher for HIV+ [0.11 (0.08, 0.15)] and DR-TB patients [0.12 (0.08, 0.17)]. We found similar CFRs for adult [0.05 (0.03, 0.08)] and pediatric [0.04 (0.02, 0.09)] patients. The public sector CFR was 0.05 (0.04, 0.07) but only 4 of 125 (3.2%) papers described privately treated patients, precluding a pooled estimate for this strata. Out of 125 studies, 78 (62.4%) had limited generalizability, 31 (24.8%) had selection bias, and 6 (4.8%) had short follow-up times.

Conclusion

Our study shows that overall, Indian TB patients experience a CFR equal to that called for in the WHO End TB strategy. However, the CFR is not well described or is unacceptably high for important vulnerable groups. This work highlights the need for more high quality patient follow-up, especially in India's large private healthcare sector.

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Poster #5

Association study of leprosy and classical HLA genes by next generation sequencing

Dallmann-Sauer M[1,2,3], Fava VM[1,2], Cobat A[4,5], Thuc NV[6], Thai VH[6], Alcais A[4,5,7], Abel L[4,5,7], Schurr E[1,2,3]

Monica Dallmann-Sauer, Postdoctoral fellow

Supervisor: Dr. Erwin Schurr

Leprosy is a chronic disease caused by Mycobacterium leprae. Worldwide, more than 200,000 new patients are affected by leprosy every year, making it the second most common mycobacterial disease after tuberculosis. Several human genetic loci have been associated with leprosy susceptibility, including several classical HLA genes. The role of HLA alleles, a combination of amino acid variations, have so far been investigated in leprosy employing low resolution methods for HLA typing. Here, we applied next generation sequencing for HLA typing with high resolution in 11 HLA genes. These alleles were used in an association study with leprosy in 1,155 individuals from a case-control Vietnamese population sample. In total, 198 HLA alleles with frequencies >1% were tested for association, and 15 alleles from the HLA-A, -C, -B, -DRB1, -DQA1, -DQB1 and -DPB1 genes reached statistical significance in an additive model. By conditional analysis we found that all associated risk alleles in the class I and class II genes belong to the same association signal, making it difficult to distinguish the HLA gene(s) primarily carrying the risk signal for leprosy susceptibility. To overcome this issue, we tested the association of leprosy with single amino-acids (AA) that were part of classical HLA genes. Out of 552 tested AA markers we found 82 to be significantly associated with leprosy. Among those, AA markers from the HLA-DRß1 protein presented the strongest association signal. We showed that two independent signals reflecting the presence of aspartic acid in HLA-DRß1 at position 57 (P = 1.81E-10) and phenylalanine in HLA-DRß1 at protein position 13 (Pcond DRB1-57D = 6.49E-6) were sufficient to explain the significant associations for both HLA alleles and amino-acid markers. These results implicated HLA-DRß1 in leprosy susceptibility and highlighted HLA protein AA changes that impact on leprosy risk.

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Poster #6

The impact of mycobacterial antigen stimulation on transcript expression in whole blood

Wilian Correa de Macedo[1,2,3], Marianna Orlova[1,2], Nguyen Van Thuc[4], Vu Hong Thai[4], Laurent Abel[5,6,7], Alexandre Alcaïs[5,6,7], Luis Bruno Barreiro[8], Erwin Schurr[1,2,3]

Wilian Correa de Macedo, PhD student

Supervisor: Dr. Erwin Schurr

Leprosy type-1 reactions (T1Rs) are a major contributor to nerve damage and disability in leprosy patients. Early recognition of patients at risk of T1R is key in the efforts to prevent nerve damage in leprosy but useful biomarkers for early diagnosis are lacking. In this study, we asked if the gene isoform (transcript) response of whole blood to Mycobacterium leprae antigens could be used as biomarker to identify patients at increased risk of developing T1R.

Samples from 32 T1R-free and 10 T1R-destined leprosy patients were stimulated with PBS or PBS+M. leprae sonicate. RNA transcript quantification was performed with SALMON v0.12, differential transcript expression (DTE) test with limma and differential transcript usage (DTU) analysis with DRIMSeq-v1.6. Gene ontology (GO) analyses were performed with PANTHER.

We first investigated the global effect of antigen stimulation and identified 6,776 transcripts up-regulated and 5,727 down-regulated implicating 5,915 genes in the response to M. leprae. Surprisingly, only up-regulated transcripts had significant GO categories (572 at FDR ≤ 0.05). This result provided evidence of a coordinated response represented by up-regulation of transcripts implicating pathways of response to sterile insult such as processing, presenting and clearing of bacteria. In DTU, 3,032 transcripts (1,702 genes) were statistically different. A sub-set of 447 DTU genes presented usage events in which lower proportion isoforms where on par or surpassed the proportion of main transcripts after stimulation. GO testing for usage event sub categories or for all DTU genes resulted in no significant categories.

DTE testing for T1R predisposition yielded no significant transcripts, while 63 transcripts (27 genes) were identified after DTU testing.

Despite the strong changes induced by antigen stimulation, T1R-destined patients did not present significant transcriptome differences from T1R-free subjects. Hence, our work failed to detect whole blood RNA transcripts that could serve as useful biomarkers for early T1R detection.

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Poster #7

Diagnostic accuracy of stool Xpert MTB/Rif for the detection of active TB in children: a systematic review and meta-analysis

Giorgia Sulis[1,2], Emily MacLean[1,2], Claudia M. Denkinger[3], James C. Johnston[4,5], Samuel G. Schumacher[3], Madhukar Pai[1,2], Faiz Ahmad Khan[2,6]

Giorgia Sulis, PhD student

Supervisor: Dr. Madhukar Pai

Background

Tuberculosis (TB) is a major cause of morbidity and mortality in children, yet its microbiological confirmation is often challenging. Xpert MTB/RIF is currently recommended as the initial diagnostic test for presumptive TB, though appropriate respiratory samples may be difficult to obtain from children. A potentially valid alternative in this population is stool, which is less invasive to collect, although standardized processing methods have not yet been defined.

Methods

We conducted a systematic review and meta-analysis according to PRISMA guidelines to evaluate the diagnostic accuracy of stool Xpert for childhood TB against (1) a microbiological reference standard and (2) a clinical reference standard. PubMed, EMBASE, Scopus, and Cochrane Library were searched for relevant publications from Jan 1, 2008 to June 15, 2018. The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool was applied to assess the risk of bias.

Results

Nine studies (involving 1681 children) were included in qualitative and quantitative synthesis. Protocols for processing and testing of stool varied substantially. Pooled sensitivity and specificity of stool Xpert versus the microbiological reference standard were 67% (95% CI:52-79) and 99% (95% CI:98-99), respectively. Sensitivity was higher among children with HIV (79%; 95% CI:68-87; versus 60%; 95% CI:44-74 amongst HIV-negative children). For the clinical standard, sensitivity was 22.0% (95%CI:9-44) and specificity 100% (95% CI:66-100). Substantial heterogeneity was observed against the microbiological reference standard, partly due to HIV status.

Conclusion

Stool Xpert could potentially be a non-invasive method of ruling-in pulmonary TB in children, particularly those with HIV; however, generalizability of the evidence base is limited by high heterogeneity and lack of a standardized stool preparation and testing protocol.

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Poster #8

Diagnostic accuracy of centralised DST assays for TB

Mikashmi Kohli[1,2], Emily MacLean[1,2], Samuel Schumacher[3], Madhukar Pai[1,2], Claudia Denkinger[3,4]

Mikashmi Kohli, Postdoctoral fellow

Supervisor: Dr. Madhukar Pai

Background

WHO estimates that in 2017, 10 million people became ill with TB globally. An estimated 558,000 people were newly diagnosed with rifampicin-resistant TB, 468,720 of whom had multidrug-resistant TB. Recently, many diagnostic companies have entered the realm of molecular testing for TB detection. These are high throughput molecular platforms which have been developed for various diseases such as TB, HIV, HPV etc. This review provides evidence on diagnostic accuracy of six of these assays.

Methods

A comprehensive search of various databases for relevant citations was performed. Various diagnostic companies were also contacted. Bivariate random-effects meta-analyses were performed using STATA to obtain pooled sensitivity and specificity estimates with 95% confidence intervals. A culture-based reference standard was used for TB detection. Resistance detection was compared against a phenotypic reference standard, sequencing and a composite reference standard.

Main results

A total of 24 studies contributed to 29 unique datasets (provided data for more than one index test). Only three of these 6 assays could be meta-analyzed as rest of the assays did not have adequate data to be analyzed. For Abbott RealTime MTB assay, a pooled sensitivity of 96% and specificity of 97% for TB detection was observed (10 studies, 4858 specimens). For Abbott RealTime RIF/INH assay, a pooled sensitivity of 94%, with a specificity of 100% for rifampicin resistance detection and a pooled sensitivity of 89% and specificity of 99% was observed for isoniazid resistance detection were observed. For FluoroType MTB assay, a pooled sensitivity of 92%, with a specificity of 99% for TB detection was observed.

Conclusion

Currently, these assays have limited data. However, all of these assays have high to moderate diagnostic accuracy for TB and drug resistance detection. Introduction of these assays will help in testing large number of patient specimens with a shorter turnaround time.

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Poster #9

Industry Perspectives on the WHO Essential Diagnostics List

Paulami Sen[1], Mikashmi Kohli[1], Madhukar Pai[1,2]

Paulami Sen, Undergraduate student

Supervisor: Dr. Madhukar Pai

Background

Quality healthcare cannot be delivered with only medicines. Patients need access to early and accurate diagnosis. On May 16, 2018, the World Health Organization (WHO) released its first "Essential In Vitro Diagnostics List" (EDL), further fostering universal health coverage. A major impact of the EDL will be felt by the diagnostics industry, whose role is pivotal in developing and supplying in vitro diagnostics. So, it is important to engage the industry to know how the EDL can be improved.

Methods

To solicit the opinions of the industry, we anonymously surveyed 30 industry representatives, using a Google Form. Data was collected during the McGill Summer Institute on Infectious Diseases and Global Health and also via email between June 11 to July 17, 2018.

Results

Most industry representatives (28/30, 93%) were aware the WHO published an EDL and believed it would be beneficial for the diagnostics field. Others (23/30, 77%) were hopeful that the EDL will improve access to diagnostics as the EML did for medicines. Yet, 53% (16/30) agreed that the EDL might have some negative consequences. Common concerns raised, in decreasing order, include: 1) Price capping (12/30, 40%) and 2) EDL revisions not keeping pace with new developments (6/30, 20%).

Conclusion

Overall, our survey suggests that the industry welcomes the WHO EDL initiative. However, industry concerns point to the fact that WHO must find a mechanism to solicit input from industry stakeholders, since they are key players in developing new technologies and in providing better diagnostics.

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Poster #10

A Novel Screening Platform for Non-Tuberculous Mycobacteria Drug Discovery

Jaryd Sullivan[1,2,3], Marcel Behr[1,2,3,4]

Jaryd Sullivan, MSc student

Supervisor: Dr. Marcel Behr

Mycobacterium abscessus (Mabs) the lesser known but closely-related species of Mycobacterium tuberculosis, the causative agent of tuberculosis (TB). In fact, Mabs does cause TB-like disease, yet has been overlooked as far as requiring tailored antibiotic therapies. As a result, we have patients with Mabs infections that have limited therapy options available. Unfortunately, the drug discovery pipeline for mycobacteria has dwindled due to their slow-growing nature, where screening techniques and validation took years of laborious experiments. In this study, we aim to develop new therapies for patients with Mabs infections by developing, and then employing, a novel screening method. We hypothesize that new therapeutics may be discovered using structural biology and chemistry to 1) develop an innovative screening platform using bioluminescence resonance energy transfer (NanoBRET), and 2) partner this platform with modifications of antibiotics on the market. First, we will design and validate NanoBRET in vitro to demonstrate feasibility. Second, we will use NanoBRET to screen for chemically modified analogs of antibiotics previously designed by collaborators at the Structure-guided Drug Discovery Coalition. We believe the unconventional approach of this project will help reinvigorate and contribute to antibiotic discovery, by providing a novel platform for screening chemical compounds and highlighting new antimicrobials. We have successfully cloned our target of interest, enoyl-[acyl-carrier-protein] reductase (InhA), from Mabs. In addition, we have expressed and purified native InhA and luciferase-tagged InhA (InhA-nluc). Luminescence assays indicate the tagged luciferase on InhA-nluc is active. Proximal work includes testing InhA activity through in vitro redox assays paired with UV-Vis spectroscopy. In parallel, we explored the cell permeability of NanoBRET tracer molecules by measuring cell viability with alamarBlue. The parent and fluorescent tracers are cell permeable and maintain cellular activity. Future experiments include producing Mabs mutants to the tracer molecules by culturing on sub-MIC concentrations to verify specific target binding.

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Poster #11

An Individual Patient Data Meta-Analysis of the Diagnostic Accuracy of an Machine Learning Software Analyzing CXRs

Abidi SK[1]*, Harris M[1,2]*, Muyoyeta M[3,4], Dheda K[5], Esmail A[5], Reither K[6,7,8], Breuninger M[6,8,9], Qi A[1], Denkinger C[10,11], Korobitsyn A[1,2], Pai M[1,2,11], Benedetti A[1,2,11], Khan FA[1,2,11]

Syed Abidi, Research assistant

Supervisor: Dr. Faiz Ahmad Khan

Background

Machine-learning is an artificial-intelligence method that is particularly promising for image analysis, but the diagnostic accuracy of machine-learning trained programs for analyzing CXRs to detect microbiologically-confirmed PTB has not been assessed.

Methods

We performed an individual patient data (IPD) meta-analysis to assess the diagnostic accuracy of a machine-learning-trained software (qXR, QURE.AI, Mumbai, India) for analyzing digital CXRs of adults presenting with symptoms of PTB, as compared to a reference standard of sputum tested with TB culture or GeneXpert. Each CXR was analyzed with qXR, which output an abnormality score ranging from 0 to 1. Pooled sensitivity and specificity were calculated using bivariate random-effects two-step IPD meta-analysis. Accuracy was assessed at an abnormality score of 0.25, 0.6, and the score with a pooled sensitivity of 0.90 was obtained.

Results

CXRs and clinical data were obtained for 1581 participants from 3 published studies, all conducted in sub-Saharan Africa. Median age was 43 (IQR: 32-75); HIV prevalence was 43.6%, and prevalence of microbiologically-confirmed PTB was 22.4%. Using 0.25 as the threshold, pooled sensitivity and specificity (95%CI) were 0.84 (0.78-0.89) and 0.64 (0.57-0.71), respectively, and between-study heterogeneity was moderate for sensitivity and substantial for specificity. With 0.6 as the threshold, sensitivity and specificity were 0.62 (0.57-0.67) and 0.89 (0.86-0.91), respectively, and heterogeneity was not substantial. A score of 0.18 achieved a pooled sensitivity of 0.90 (0.79-0.96) with a pooled specificity of 0.54 (0.46-0.62), between-study heterogeneity was substantial for sensitivity and for specificity.

Conclusion

In high HIV prevalence populations with a high pre-test probability of PTB, a machine-learning based CXR-analysis software could detect microbiologically-confirmed PTB with high sensitivity and moderate specificity, but with important between-study heterogeneity.

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An Outsider's View of Virulence: Understanding ESAT-6 in Mycobacterium kansasii

Jordan Kruisselbrink[1], Marcel Behr[1]

Jordan Kruisselbrink, MSc student

Supervisor: Dr. Marcel Behr

Mycobacterium tuberculosis uses early secreted antigenic target 6-kDa (ESAT-6), a key virulence factor, to lyse phagosomal membranes which leads to the death of infected host cells. As M. tuberculosis is a human pathogen, it follows that ESAT-6 evolved to provoke these responses. Environmental Mycobacteria such as Mycobacterium kansasii contain functional esxA, the gene encoding ESAT-6 and these bacteria evolved without the human host. This begs the question: How do the versions of ESAT-6 from M. tuberculosis and M. kansasii differ, and what does ESAT-6 do in an environmental bacterium?

We hypothesize that ESAT-6 function differs between M. tuberculosis and M. kansasii. Several of our hypotheses include 1) differential secretion levels, 2) different partners that lyse phagosomal membranes, 3) a different role, such as distributive conjugal transfer (DCT), a method of mycobacterial conjugation.

We will compare knock-out mutants of esxA in M. tuberculosis and M. kansasii with their Wild-Type counterparts. We have a deletion mutant in M. tuberculosis and are creating the deletion mutant in M. kansasii using recombineering to replace the gene with a hygromycin resistance cassette. To excise esxA, a destination vector containing the hygromycin cassette surrounded up and downstream by 500 bp of homologous DNA surrounding EsxA is being constructed with Gateway, using Mycobacterium smegmatis to validate the technique. The completed vector will be electroporated into M. kansasii and recombineering will facilitate the substitution of hyg for esxA.

Potential destination vectors have been constructed in M. smegmatis and are being validated. Potential vectors are being completed in M. kansasii and will be validated once complete.

In conclusion, a knock-out mutant of esxA is being constructed in M. kansasii, by substituting the gene for hygromycin resistance, with M. smegmatis to validate techniques. Once the mutant is created, further functional comparisons will be undertaken to address the abovementioned hypotheses.

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Poster #13

Can BCG-mediated protection be vertically transmitted?

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Humans are the only host for Mycobacterium tuberculosis (Mtb), which is the causative agent of tuberculosis (TB) and kills around 1.6 million people every year. BCG is the only licensed vaccine against TB and it is given to 80% of the newborn babies in high-endemic countries. While intradermal BCG-vaccination protects against the disseminated form of TB in children, it does not provide protection in adults. Importantly, it has been well documented that BCG-protection in children is not limited to TB, as BCG-vaccinated children are also protected against a range of pulmonary infectious diseases and sepsis. Additionally, recent epidemiological studies suggest that the protective signature of vaccinated individuals can be vertically transmitted to their progenies. Thus, we aim to test this possibility using a mouse model of BCG-vaccination.

We have recently demonstrated that systemic administration of BCG leads to epigenetic reprogramming of hematopoietic stem cells to generate protective monocytes/macrophages against Mtb, which represents a novel vaccination approach against pulmonary TB. Based on these observations, we hypothesize that systemic BCG-vaccination will epigenetically alter the germ cells in hosts that can then vertically transmit these protective signatures to the offspring.

To investigate vertically-transmitted effects of BCG-vaccination, we vaccinate 6-week old male and female mice with BCG intravenously (1x106CFU) or PBS (control). After 4 weeks, BCG-vaccinated or PBS-control mice were paired for breeding. The offspring of the BCG-vaccinated or PBS-control mice was then subjected to Mtb (aerosolized H37Rv; 50 CFU) or Influenza A virus (IAV; intranasal 90 PFU) infections. Offspring of the parental BCG-vaccinated mice did not show any protection against subsequent Mtb as assessed by lung bacterial burden or IAV infection as evaluated by survival. These results collectively suggest that the protection of parental systemic BCG-vaccination was not vertically transmitted to the offspring and further investigation is required to experimentally test this possibility.

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Leveraging Drug Discovery for Nontuberculous Mycobacteria Using Novel Anti-Tuberculosis Drug Target

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Nontuberculous mycobacteria (NTM) are emerging opportunistic pathogens that cause chronic, tuberculosis (TB)-like lung disease. One of the most clinically relevant species is Mycobacterium abscessus having the most detrimental impact on lung function and patient outcome. The success rate of treatment with current antibiotics is low (50% or less). This study aims to discover new drugs against this species, where we hypothesize that orthologues of TB drug targets would offer the most direct path to new antimicrobial therapies for NTMs. We used a combination of CRISPRi and chemistry to test whether dihydrofolate reductase (DHFR) would be a valid target for the treatment of M. abscessus.

In preliminary work, the CRISPRi methodology has been first validated in the model organism M. smegmatis and subsequently in M. abscessus. With this technique, the DHFR gene, dfrA, was observed to be essential for in vitro growth of two subspecies of M. abscessus. A series of candidate DHFR inhibitors has been tested for in vitro growth inhibition using broth microdilution and conventional agar dilution method. A few of these showed potent antimicrobial activity against M. abscessus (MIC90 at 10 µM or lower).

Together, these data suggest that DHFR can serve as a promising drug target for NTM infections, as shown via CRISPRi and chemical inhibition for M. abscessus. Ongoing studies aim to determine whether the use of efflux pump inhibitors may potentiate activity of our candidate DHFR inhibitors. The validation of this target in another clinically relevant NTM species, M. avium, is also currently under investigation.

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The Cost of Multidrug-Resistant Tuberculosis in Canada

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Background

Multidrug-resistant tuberculosis (MDR-TB) requires prolonged treatment, expensive drugs, and close monitoring for treatment side effects. The costs of providing this care; however, are largely unknown. We estimated the health system costs for patients who completed MDR-TB treatment in British Columbia, Ontario, and Quebec, Canada.

Methods

We chart-reviewed all MDR-TB patients who initiated treatment at the British Columbia Centre for Disease Control (Vancouver), West Park Healthcare Centre (Toronto), and Montreal Chest Institute (MCI) between January 2010 and June 2016 and subsequently completed treatment. Information regarding consumables (e.g. drugs, supplies), services, and personnel time used during diagnosis, treatment, and follow-up was extracted. Quebec costs for each item were used to estimate the total cost per MDR-TB episode in all settings. For comparison with MDR-TB, we also calculated overall costs for 30 patients treated at the MCI for drug susceptible TB (DS-TB) and latent TB infection (LTBI).

Results

Forty-six MDR-TB patients were included. Mean age was 35 (SD 13.4) years, 25 patients (54%) were female, and 4 (9%) had extensively drug-resistant TB. The most common additional resistances were to rifabutin (87%), streptomycin (72%), pyrazinamide (57%), and ethionamide (30%). Across all sites, the median (Q1, Q3) cost of MDR-TB was $124 969 ($98 821, 162 403). The costliest aspects of MDR-TB care were treatment ($71 029) and hospitalization ($42 147), correspondingly accounting for 57% and 34% of costs. Cycloserine was the most expensive medication, taken by 28 (61%) patients and costing $51 111 per person on average. The costs of DS-TB and LTBI were <5% the costs of MDR-TB. The median (Q1, Q3) costs for DS-TB and LTBI were $4736 ($3605, $15 972) and $834 ($791, $929), respectively.

Conclusion

MDR-TB is very expensive to treat and manage in Canada. The high costs of MDR-TB appear to be driven by hospitalizations and costly drugs.

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Country adoption and uptake of urine LAM test: current landscape and barriers

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The lateral flow lipoarabinomannan (LF-LAM) TB test was recommended by WHO in 2015 for use in screening for TB in PLHIV whose CD4 T-cell count is below 100 cells/µl. However, anecdotally, few countries are known to have implemented the use of the LF-LAM assay in their HIV or TB national program. We are therefore conducting a study in 31 high TB and HIV/AIDS burdened countries to assess the current landscape, and to determine the barriers to the adoption of LF-LAM.

A semi-structured questionnaire was sent to the National TB or HIV/AIDS programs managers. The current response rate is approximately 45%, but we expect more surveys to be completed since the study is still ongoing. Based on the feedback, 5 of 14 countries have included LF-LAM in their national policies and of these, 3 are currently using LF-LAM. An additional X countries are considering adoption in the near future.

Our preliminary results indicate that budget limitation is a barrier to LF- LAM adoption, followed by a lack of evaluation/pilot studies and a low priority due to the relatively small size of the affected population. Challenges with the product registration process, lack of buy-in and the absence of LF-LAM's in the national TB or HIV programs' mandate were also cited as hurdles in FL-LAM implementation.

When LF-LAM is used and/or included in the national policy it is incorporated in the diagnostic algorithm for adult in-patients. In only one case did the respondent state that the test could be used in an ambulatory setting. Similarly, there were only two countries that extended LF-LAM testing to children as part of their algorithm. Since the national roll out of the test is very recent or still in an evaluation phase, it is only available in a limited number of hospitals or treatment centers.

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How resource intensive is latent TB management? Using time and motion studies to estimate labour needs for LTBI scale-up

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Background

Time and motion (TAM) studies have been used to precisely quantify the time required for specific work activities, such as assembly line workers. We have used TAMs in a novel way: to quantify the increase in healthcare workers (HCW) time spent on management of latent tuberculosis infection (LTBI) following LTBI program strengthening (ACT4 Trial).

Methods

HCW involved in TB care at the 24 ACT4 health facilities were invited to participate. Those who agreed were followed for a full work day, noting each of their daily activities, which were quantified into pre-determined categories such as LTBI services. To assess changes in their workload, HCW workers were followed before and after the intervention. Based on the number TB patients treated at ACT4 facilities, increased time on LTBI was extrapolated regionally to estimate total work-force time required for LTBI program scale-up.

Results

A total of 140 HCW in five countries participated in the baseline TAM (before LTBI program strengthening). Data was available for 106 of these HCW after the intervention was implemented. For these workers there was a 10% increase in the proportion of time spent on LTBI-related activities at intervention sites on average, corresponding to an additional 40 minutes per work day.

Conclusions

We found that there has been a significant increase in the proportion of HCW time spent on LTBI-related activities in ACT4 intervention sites. This increased workload can be extrapolated to estimate workforce requirements following similar LTBI programme strengthening and expansion in other settings.

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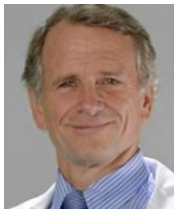

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The McGill International TB Centre (www.mcgill.ca/tb/) is a world leader in the interdisciplinary study of TB. This Centre brings together over 20 investigators with expertise ranging from public health to mouse models, working both at an academic centre and with a number of collaborating groups around the world. The Centre includes researchers interested in biomedical, clinical, epidemiologic and social determinants of TB. Their work aims to develop and evaluate new diagnostic tests, new vaccines and new treatment regimens for the control of TB and other mycobacterial diseases.