Methods & Materials: Ten-fold reductions of whole MTB (10⁴ to 1 CFU/mL) in PS-MTM and PBS control were analyzed using GeneXpert and real-time PCR on an ABI 7500. For Xpert evaluation of clinical material, 50-150 μL of smear-positive (3+ to 1+) sputum specimens (N = 17) were transferred by flocked swab into PS-MTM and compared to equivalent amounts collected in PBS and routine Xpert testing according to manufacturer's recommendation.

Results: Using Xpert, MTB from PS-MTM was detected at 10 CFU/mL compared to 10² CFU/mL MTB detected from PBS controls. Overall Xpert PCR efficiency from PS-MTM (63.2%) was improved compared to PBS controls (34.9%). In Xpert, CTF values from higher MTB concentrations in PS-MTM were increased compared to control; however PS-MTM showed superior detection from low level MTB concentrations. Xpert assay detected MTB from sputum collected by flocked swabs placed in PS-MTM in 17 of 17 specimens and corresponded to routine Xpert detection using 1.0 mL of sputum. NGS of multi-drug resistance genes was performed from the volume remainder of five PS-MTM specimens. Resistance conferring mutations for rifampicin were noted from two specimens in the rpoB gene, which corresponded to Xpert rifampin-resistance detection.

Conclusion: PS-MTM enhances MTB detection when specimens contain low level MTB. Sputum collection in PS-MTM provides safe and inexpensive shipment/transport at ambient temperature to centralized testing sites. Small sputum volume collected using a flocked swab allows the remaining sample to be safely archived, re-tested, or evaluated for drug resistance by NGS.

http://dx.doi.org/10.1016/j.ijid.2016.02.840

Type: Poster Presentation

Identification of Mycobacterium tuberculosis complex in clinical specimens of HIV-infected patients at Instituto de Infectologia Emilio Ribas, Sao Paulo-Brazil

M.D. Eira 1,∗, E. Boccardo 1, R.J. Costa Silva 2, U. Barbosa 2, I. Moreira 1, S.A. Souza 3, F.I. Oliveira Junior 3

1 Instituto de Infectologia Emilio Ribas, São Paulo, SP, Brazil
2 Instituto de Infectologia Emilio Ribas, Arak, Iran
3 Instituto de Infectologia Emilio Ribas, São Paulo, Brazil

Background: Tuberculosis (TB) remains the most common infection among HIV patients. Currently, the TB diagnosis is still based on the clinical presentation, radiographic findings and microbiological results. Considering the complexities of treating HIV/TB co-infection, TB diagnosis requires the availability of diagnostic tools that allow the rapid detection of Mycobacterium tuberculosis complex (MTBC) and drug resistance in clinical samples.

Methods & Materials: In this retrospective study conducted at the Instituto de Infectologia Emilio Ribas, São Paulo/BR, we analyzed a total of 5350 clinical specimens (respiratory and extrapulmonary) collected from patients with signs and symptoms suggestive of TB from January/14 to December/14. All samples were processed by conventional diagnostic techniques, including smear examination for acid-fast bacillus (AFB) and cultured in MGIT 960 automated system. Blood and bone marrow were cultured in BACTEC FX. Identification of MTBC and non-tuberculous mycobacteria (NTM) was performed by rapid immunochromatographic assay. The average time needed for detection of mycobacteria was 15 days. Susceptibility testing for MTBC and PCR for NTM was performed by Adolfo Lutz Institute, S. Paulo.

Results: Of the 5350 samples, 554 (10.35%) were positive by culture for mycobacterial agents. Among the culture positive specimens, 428 (77.25%) were from HIV-infected patients, and 342 (61.73%) were collected from male. From the 554 culture-positive specimens, of which 398 (71.84%) respiratory and 156 (28.15%) extra-pulmonary, 391 (70.57%) had a positive rapid test for MTBC and 95 (17.14%) had a positive rapid test for NTM. From NTM, Mycobacterium avium complex (MAC) was the most prevalent (52.48%), followed by M. kansasii (15.78%) and M. fortuitum (5.26%). Resistance was identified in 25/391 MTBC isolates (6.3%), and the most frequently resistant drugs were rifampin (44%) and isoniazid (32%), respectively.

Conclusion: TB is an important public health problem and the diagnosis in HIV-infected patients is challenging. The use of mycobacterial culture remains an important diagnostic tool. The immediate future involves rapid molecular techniques, in particular GeneXpert which is also able to detect rifampicin resistance. In order to improve diagnosis and detect as early as possible resistance to rifampin, it was introduced earlier this year the GeneXpert MTB/RIF in our hospital which proposes to be a strong diagnostic tool for pulmonary TB.

http://dx.doi.org/10.1016/j.ijid.2016.02.841

Type: Poster Presentation

Cholecalciferol adjunctive therapy in active tuberculosis

A. Farazi 1,∗, F. Didgar 2, M. Jabbariasi 2, A. Sarafraz 2

1 Arak University of Medical Sciences, Arak, Markazi, Iran, Islamic Republic of
2 Arak University of Medical Sciences, Arak, Iran, Islamic Republic of

Background: Vitamin D enhances immune responses to tuberculosis bacillus. The aim of our study was to demonstrate whether or not use of cholecalciferol as supplement to patients with TB may be improved clinical outcome.

Methods & Materials: Sixty patients with pulmonary tuberculosis were randomised to take either 450000 International Units of cholecalciferol or placebo. Evaluation were carried out at one, two and three months later. The first outcome was reduction in TB score and the secondary outcome was smear conversion and improvement of quality of life. Analyses were conducted using SPSS software (ver. 18) according to a pre-specified plan.

Results: Mean calcidiol levels for the whole study population were within the insufficient range (22.81 ± 10.76 ng/ml). There have been no associations between baseline calcidiol lev-
els and sputum smear burden (P-value = 0.54). There was an association of TB severity score with lower levels of Vitamin D (P-value = 0.043). The general social functioning (SF)-12 health survey scoring at enrolment in two arms did not differ significantly (P-value = 0.786). However two months later findings indicate that 25-hydroxyvitamin D treatment had a positive effect on progressing health-related quality of life (P-value = 0.019) in each subscale of physical health score (P-value = 0.028) and mental health score (P-value = 0.025).

**Conclusion:** Our findings indicated that high dose cholecalciferol supplementation can lead to improve clinical outcome in tuberculous patients especially in patients with calcidiol deficiency. Tuberculosis alleviate quality of life and necessary at TB clinics to apply strategies to improve the health-related quality of life of TB patients. Therefore, we recommend vitamin D supplement therapy for this purpose.

http://dx.doi.org/10.1016/j.ijid.2016.02.842

**Type:** Poster Presentation

**Final Abstract Number:** 43.105

**Session:** Poster Session III

**Date:** Saturday, March 5, 2016

**Time:** 12:45-14:15

**Room:** Hall 3 (Posters & Exhibition)

---

**Mycobacterium tuberculosis acetyltransferase reduces the oxidative stress response through expression of peroxisomal membrane transporter protein**

G. Ganguli1, 2, A. Sonawane2

1 KIIT University, Bhubaneswar, Orissa, India
2 KIIT University, Bhubaneswar, Odisha, India

**Background:** Mycobacterium tuberculosis (M.tb) survives inside the macrophages by manipulating the host immune responses. Mtb cell-wall associated glycoproteins play an important role in initiation of host-pathogen interactions.

**Methods & Materials:** M.smegmatis mc2155 was used in this study. Cloning and expression was performed in pSMT3 shuttle vector. Invasion assay in HeLa cells. Survival assay, autophagy, oxidative stress response, immunostaining by fluorescence microscope and Western blot analysis was done in mouse macrophages. Microbial adhesion to hydrocarbons (MATH) test was performed to assess bacterial hydrophobicity. Bacterial susceptibility against cell wall acting antibiotics was done with CFU (Colony Forming Unit) assay.

**Results:** To identify novel Mtb glycoproteins, we employed a multi-lectin system to capture glycoproteins from purified Mtb cell wall and identified them by mass spectrometry analysis. A novel protein as putative acetyltransferase (AcTase) was identified. Recombinant M.smegmatis expressing the ACTase (MsmAcTase) showed increased invasion in human epithelial cells and survival in mouse macrophages. Increased intracellular bacillary burden was a result of inhibition of autophagy and ROS production due to reduced expression of superoxide dismutase (SOD) and catalase enzymes in Msm AcTase infected macrophages when compared with wild-type and vector control (pSMT3) strains. Subsequent studies showed that decreased ROS production was due to over expression of ROS scavenging peroxisomal membrane protein 70 (PMP70). MsmAcTase showed increased expression of acylCoA oxidase (ACOX-1), a classical marker enzyme for peroxisomal β-fatty acid oxidation. MsmAcTase also exhibited increased production of nitric oxide and expression of inducible nitric oxide synthase (iNOS) in infected macrophages. Moreover, MsmAcTase showed increased resistance to cell wall acting anti-TB drugs and to lysozyme due to the increased cell surface hydrophobicity.

**Conclusion:** We have shown that acetyltransferase gene (ACTase) of Mtb expressed in M.smegmatis aid intracellular mycobacterial survival through inhibition of autophagy and oxidative stress responses in macrophages. The present study reports for the first time that MsmACTase scavenges H2O2 due to over expression of ROS scavenging peroxisomal membrane protein 70 (PMP70) with which insights a new mechanism how the pathogen surpass the host defense in Mtb infection. The above findings may lead to identification of a potential drug target for the antimycobacterial therapy.

http://dx.doi.org/10.1016/j.ijid.2016.02.843

**Type:** Poster Presentation

**Final Abstract Number:** 43.106

**Session:** Poster Session III

**Date:** Saturday, March 5, 2016

**Time:** 12:45-14:15

**Room:** Hall 3 (Posters & Exhibition)

---

**Analysis on direct medical costs and compensation for whole course of treatment of pulmonary tuberculosis patients in Shanghai**

M.-L. Guo1, 2, W. Wang2, X. Shen3, J. Chen3, Z. Yuan4, F. Yan2

1 School of Public Health, Fudan University, Shanghai, China
2 Fudan University, Shanghai, China
3 Shanghai Municipal Centre for Disease Prevention and Control, Shanghai, China
4 Shanghai Center for Disease Control and Prevention, Shanghai, China

**Background:** To describe the overall direct medical costs and the compensation from health insurance systems and the TB control projects, and analyze the economic burden for the whole course of treatment of pulmonary tuberculosis (PTB) patients in Shanghai.

**Methods & Materials:** The copy of invoices during the course of TB diagnosis and treatment of 766 newly registered PTB patients from 2013 were collected in four districts in Shanghai, meanwhile these patients were investigated by questionnaire. Descriptive analysis and ranksum test were employed.

**Results:** The medians (inter-quartile range) of 766 PTB patients’ direct medical costs for whole course of treatment were 5757.4 (3749.87, 12632.98) yuan/person. All patients had an out-patient cost with a median of 4605.3 (3490.8, 6335.2) yuan/person, consisted of the cost of western medicine (47.6%), Chinese patent drugs (19.9%), laboratory test fee (18.1%), inspection fee (12.1%) and treatment/registered fee (2.3%). Among the western medicine fee, hepatoprotectants accounted for 57.1%, followed by the first-line anti-TB drugs (20.2%) and second-line anti-TB drug (11.1%); a higher proportion of hepatoprotectants in Chinese patent drugs, reached 60.8%; in laboratory test fee, costs of liver function tests (36.2%) and tuberculosis determination (31.5%) were relatively higher; inspection fee was mostly CT cost, accounting for 82.9%. The amount of cost was related to the District, age, the location of household register, health insurance types, the level of medical institutions, category of PTB. The median of the proportion of health...