435. Novel strategies for the diagnosis of tuberculosis

P4380
Late-breaking abstract: Expression of IFN-g/IL-10 in active pulmonary tuberculosis patients and household contacts
Sumanlatha Gaddam[1], Lavanya Joshi[1], Hari Sai Priya Vanacherla[1], Anuradha Bandaru[2], Vijayalakshmi Valluri[1,2]. [1]*Immunology, Bhagwan Mahavir Medical Research Centre, Hyderabad, Andhra Pradesh, India;* [2]*Immunology and Molecular Biology, LEPRA- Blue Peter Research Centre, Hyderabad, Andhra Pradesh, India*

There is an increase in the prevalence of tuberculosis in young adults. Since,cytokine mediated immune responses to *Mycobacterium tuberculosis* infection are important determinants of disease development and pathology, the aim was to investigate the role of candidate cytokines in active pulmonary tuberculosis patients (APTB) of younger age (15to25yrs) & their HouseHold Contacts (HHC). T cell assays were stimulated with r32-kDa antigen of *M.bovis* BCG (Ag85A-BCG), IFN-g & IL-10 were measured in the culture supernatants by ELISA in APTB (15), HHC (PPD-positive) (15) & Healthy Controls (HC) (PPD status not known) (15). Expression levels were determined by quantitative real time-PCR in 4 individuals from each group. The mean proliferative responses of stimulated cells were significantly low ($p<0.05$) in APTB and HHC compared to HC (1.35±0.72; 1.55±0.89 and 4.48±4.43) respectively. The mean IFN-g ($p<0.05$) (43.4+24.8; 46.0±22.2 &70.9±41.5) & IL-10 (85.7±58.7; 63.0±44.2 & 11.4±7.7 pg/ml ($P<0.003$ &<0.0006) levels were significantly low and high respectively in APTB & HHC when compared to HC. The expression of IFN-g was high (5-fold) in HC when compared to APTB (1.5-fold) &HHC (3-fold), against the corresponding unstimulated cells. IL-10 expression increased by 8- fold in APTB & 6-fold in HHC compared to HC (2-fold). In conclusion, elucidation of the mechanism by which Th1 cytokine is down-regulated may enhance our understanding of susceptibility to disease. Also, follow-up of the contacts for their clinical status may help in identifying a biomarker for house-hold contacts useful for early diagnosis.

P4381
Analysis of C-reactive protein and fibrinogen as possible predictors of secondary fibrosis in pulmonary tuberculosis
Besim Prnjavorac[1], Adlija Cauševic[2], Sabina Semiz[2], Tanja Dujic[2], Tamer Bego, Nedzada Ireijz[1], Rifat Sejdinovic[1]. [1]*Department of Lung Disease, General Hospital Tešanj, Tešanj, Bosnia and Herzegowina;* [2]*Clinical Biochemistry, Faculty of Pharmacy, Sarajevo, Bosnia and Herzegowina*

Aim: To study the influence of C-reactive protein (CRP) level in the blood, fibrinogen level and general inflammatory syndrome as the predictors of development of secondary fibrosis in patients with pulmonary tuberculosis (TB).
Methods: Concentration of CRP, fibrinogen level was measured using immunoturbidimetric methodIncluding criteria was presentation of TB process in both lungs, as the sign of widespread TB process.
Results: We examined 85 patients treated in one year. Mean CRP level was 22,6 mg/mL, range 5-245 mg/mL; normal level (up to 8 mg/mL) was measured in 23,4% patients, medium level (9-20 mg/mL) was measured in 31,3% patients, high level (21-50 mg/mL) were measured in 26,2% patients, and in 23,7% patients CRP were higher than 50 mg/mL. Average fibrinogen level in whole group was 6,9 g/L (SD 5,8). Normal level of fibrinogen (up to 4 g/L) were measured in 6,4% of patients; 4,1-1,0 g/L were measured in 24,6% patients, 10,1-20 g/L were measured in 31,1% patient and level more than 20 g/L were measured in 37,9% patients. Using statistic method of partial correlation statistical significane at level $p<0,05$

was shown between them. Correlation of CRP and fibrinogen level with appearance of fibrosis on X-ray of the lung was shown. Thereafter, closer correlation was shown with fibrinogen and fibrosis than with CRP and fibrosis.
Conclusion: Predicted value of CRP and fibrinogen for pulmonary fibrosis was shown in TB patients. So, attenuation of fibrosis development, possible with antifibroblastic activity of pentoxyphyllin, should be taken in consideration, for prevention of widespread development of lung fibrosis in these patients.

P4382
Decreasing level of serum ADA: A valuable predictor of treatment in smear positive tuberculosis
Kazem Navari[1], Tasnim Eqbal Eftekhaari[2], Najme Ziaee[1], Farzaneh Navari[1].
[1]Internal Medicine, Hormozgan University of Medical Sciences, Shahid Mohammadi Hospital, Bandar Abbas, Hormozgan, Islamic Republic of Iran; [2]Infectious Disease Research Department, Research Department, Hormozgan University of Medical Sciences, Shahid Mohammadi Hospital, Bandar Abbas, Hormozgan, Islamic Republic of Iran

Background: High titer of adenosine deaminase (ADA) in pleural effusion is an indicator of pleural tuberculosis and necessity of treatment initiation. On the other hand high serum titer of ADA is a characteristic of tuberculosis, and is used for treatment follow up in patient on standard antituberculosis regimen. Decreasing level of ADA could be a predictor of treatment response.
Methods: A cross sectional study was carried out on 29 patients from Sep 2007 till Dec 2010. All of the tuberculosis patients included had a positive sputum smear or positive biopsy or bronchoalveolar lavage (BAL). ESR and ADA were checked for these patients in treatment initiation, 30th and 60th day of treatment. According to ADA diagnostic kit values, ADA more than 15 is high.
Results: Mean age of the patients was 38.9 years. Mean ADA before therapy was 19.31 which gradually decreased to 12.37 (on day 30th) and 11 (day 60th). Mean ESR before initiation of therapy was 65 which decreased gradually to 38.66 (day 30th) and finally 23.28. Male patients were 55.2%, 82.8% suffered from pulmonary TB. Comparison of ADA and ESR at the end of therapy showed a significant difference (p=0.000). Mean ADA (60th day) in males was 11.18±1.60 and in females were 10.76±0.42. Mean ESR (60th day) in males was 25.50±5.48 and in females was 23.00±2.02
Conclusion: Decreasing level of serum ADA is a valuable and reliable predictor in successful treatment of tuberculosis.

P4383
Adenosine deaminase, an useful tool for the diagnosis of tuberculous pleuritis in France
Hervé Le Floch[1], Sandrine Duron[2], Hervé Delacour[3], Frédéric Rivière[1], Inès Ngampolo[1], Fabien Vaylet[1], Jacques Margery[1], Christophe Rapp[4].
[1]Respiratory Diseases, Military Hospital Percy, Clamart, France; [2]Epidemiology Department, Military Hospital Bégin, Saint Mandé, France; [3]Biochemistry Laboratory, Military Hospital Bégin, Saint Mandé, France; [4]Infectious and Tropical Diseases, Military Hospital Bégin, Saint Mandé, France

Pleural tuberculosis is a diagnosis challenge for usual mycobacterial analysis given that the bacillary concentration is low in the pleural effusion. Adenosine deaminase is a pleural inflammatory marker recommanded for the diagnosis of pleural tuberculosis in the high-prevalence countries. There is not enough data to support its use in a low-prevalence country.
Objective: To know the utility of ADA for the diagnosis of pleural tuberclosis in France, a low prevalence country.
Material an method: Retrospective study of the exsudative pleural effusion with the ADA dosage (Giusti's method) done in two military hospital near Paris. We compared tuberculous and non-tuberculous pleural effusion. The best cut-off value of ADA for the diagnosis of pleural tuberculosis was found using ROC curves.
Results: 183 patients were studied, including 29 tuberculosis, 65 cancers, 5 malignant hemopathies, 32 parapneumonic pleural effusions, 24 purulent pleuritis, 12 old transudative effusions, 14 effusions from others aetiologies. Sixty-eight effusions were rich of lymphocytes including 23 tuberculosis. The young age (≤35 years-old), the foreign origin and the presence of general manifestations were independanltly associated with tuberculosis. The best cut-off value for ADA was 47 UI/l, with Se: 95,7%, Spe: 91,1%, PPV: 84,6%, NPV: 97,6%. When ADA ≥ 70 UI/l, the PPV was 90,5%.
Discussion: ADA dosage is usefull in France. Lowers than 47 UI/l, it may exclude tuberculosis. When ADA ≥ 47 UI/l, the result should be interpretated considering the context and the pre-test probability of tuberculosis. When this pre-test probability of tuberculosis is important, ADA ≥ 70 UI/l is very likely for tuberculosis.

P4384
Clinical utility of a lateral flow serologic test in the rapid diagnosis of pulmonary TB in a public-private mix for DOTS setting in Iloilo City, Philippines
Dennis Dumaplin[1], Malbar Ferrer[1], Ella Mae Divinagracia[1], Lorelei Sirilan[1], Rosauro Cabana[1], Rodrose Guzman-Trivilegio[1], Amee Lourdes Ponje[1], Elcie Solis[2], Peter Bretan[3], Jessica Adamich[3]. *[1]Internal Medicine, Saint Paul Hospital, Iloilo City, Iloilo, United States;[2]DOTS-PPMD, Saint Paul Hospital, Iloilo City, Iloilo, United States; [3]College of Osteopathic Medicine, Touro University, Iloilo, CA, United States*

Background & rationale: Diagnosis of PTB in a developing country with a limited resource relies largely on clinical features, sputum exam and chest xray. In an endemic area such as the Philippines, the urgent implementation of intensified case-finding and infection control measures which reduce the burden of TB is essential to saving lives. Culture methods are not routinely used in the locality due to the low cost-effectiveness and time constraints. By using serologic methods, the time required to reach a clinical decision to treat a suspected case of TB may be significantly reduced. We have investigated the utility and diagnostic accuracy of a lateral flow serologic test in PTB diagnosis when used as an adjunct in the PPM-DOTS setting.
Methods: An immuno-chromatographic TB STAT PAK II Assay was employed for the detection of antibodies to M. tuberculosis in the human whole blood of TB suspects. Humoral response was analyzed in a group of 105 TB suspects (74 in the active PTB group - 47 smear-positive and 31 smear-negative, and 31 in the non-active/control group - 31 smear-negative and 6 healthy subjects.
Results: The proportion of all test subjects with PTB who tested positive with the assay was 65%, while the proportion of all subjects without PTB who tested negative was 100%. The positive predictive value (PPV) and the negative predictive value (NPV) was 100% and 50.94%, respectively.
Conclusion: The serologic test performed with excellent specificity and acceptable sensitivity in PTB diagnosis in an endemic setting, though not enough evidence exists that they perform well enough to replace sputum microscopy.

P4385
Prognostic values of serum IP-10 and IL-17 in patients with pulmonary tuberculosis
Yung-Che Chen[1], Meng-Chih Lin[2], Chung-Jen Chen[3], Chien-Hung Chin[1], Shih-Feng Liu[1], Chao-Chien Wu[1], Chia-Cheng Tsen[1], Yi-Hsi Wang[1], Tung-Ying Chao[1], Chien-Hao Lie[1], Chin-Chou Wang[1]. *[1]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Chang Gung Memorial Hospital-Kaohsiung Medical Center, Chang Gung University College of Medicine, Kaohsiung, Taiwan; [2]Division of Pulmonary and Critical Care Medicine, Xiamen Chang Gung Hospital, Xiamen, China; [3]Division of Rheumatology, Chang Gung Memorial Hospital, Kaohsiung, Taiwan*

Objective: To identify patients at high risk of relapse after anti-tuberculosis (TB) therapy or with poor outcomes.
Methods: Fifty-one patients with pulmonary TB: 7 were classified as high risk of relapse (HR, both cavitations on initial chest radiography and positive sputum smear/cultures after two months of anti-TB treatment); 19 medium risk (MR, one risk alone); and 25 low risk (LR, neither risk). Serum interferon (IFN)-γ-inducible protein 10 (IP-10), and interleukin-17 (IL-17) levels were investigated.
Results: There was a trend towards higher serum IP-10 levels (p=0.042) for HR patients throughout the 6-month treatment period. Month-2 IP-10 levels were higher in the HR than in the MR/LR group (p=0.005). The risk of relapse was well-captured by month-2 IP-10 levels.

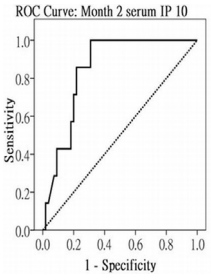

Figure 1. Predictive accuracy of the serum IP-10 levels measured at month 2. The corresponding ROC curve showed that the risk of relapse was well captured by month-2 IP-10 levels at a cut-off value of 431 pg/ml (AUC = 0.857, 95% CI 0.75-0.97, p = 0.003).

Month-2 serum IL-17 levels were lower in non-survivors than survivors (p=0.001). Multivariate analysis demonstrated that a month-2 serum IL-17 level of ≤17 pg/ml (p=0.026) was independently associated with all-cause mortality.

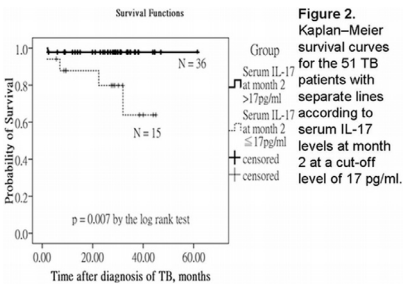

Figure 2. Kaplan–Meier survival curves for the 51 TB patients with separate lines according to serum IL-17 levels at month 2 at a cut-off level of 17 pg/ml.

Conclusions: Serum IP-10 and IL-17 levels after 2 months of anti-TB treatment may be surrogate markers for predicting risk of relapse and mortality, respectively.

P4386

IP-10 is an additional marker to evaluate the RD1-specific responses in HIV-infected subjects

Valentina Vanini[1], Teresa Chiacchio[1], Cristiana Gioia[1], Gilda Cuzzi[1], Nicoletta Orchi[1], Alessia Rianda[2], Lucia Alba[2], Maria Letizia Giancola[2], Aristide Conte[2], Elisa Petruccioli[1], Linda Petrone[1], Enrico Girardi[1], Delia Goletti[1]. *[1]Epidemiology and Preclinical Research, INMI, Rome, Italy; [2]Clinical Department, INMI, Rome, Italy*

Background: The suboptimal sensitivity of IFN-γ-based assays, especially in immunocompromised individuals, emphasizes the need for alternative markers for diagnosing tuberculosis (TB). Objective of this study was to evaluate whether IP-10 can be a useful biomarker for evaluating a specific response to RD1 antigens associated to active TB in HIV-infected individuals. Control with QuantiFERON-TB Gold In tube (QFT-IT) was performed.
Methodology: 118 HIV-infected individuals were prospectively enrolled in Rome, 21 with active-TB and 98 without. Epidemiological characteristics were analyzed. IFN-γ and IP-10 response to QFT-IT was performed. Plasma was harvested at day-1 and soluble factors evaluated by ELISA.
Results: Significant differences between those with or without active TB were found for the CD4+ T cell counts (p=0.02), and IFN-γ and IP-10 response to QFT-IT (p=0.001 for both analysis). Differently no significant differences were found for the age and HIV-RNA. Based on the commercial cut-off of the QFT-IT and on a cut-off found by ROC analysis for the IP-10-based responses, the sensitivity for active TB of QFT-IT and the IP-10 to QFT-IT was 52% and 67% respectively (p=0.001; K: 0.545). The response to IP-10 was not influenced by the ability to respond to the mitogen. The specificity for active TB of QFT-IT and of the experimental test were 84% and 77% respectively (p=0.01; k:0.710). Among those without active TB a significant correlation between a positive score and Mtb exposure was found (p<0.001).
Conclusions: These data suggest that IP-10 is an additional marker to evaluate the RD1-specific responses in HIV-subjects confirming data previously obtained in high TB endemic countries.

P4387

Evaluation of platelet count and indices in pulmonary tuberculosis and pneumonia

Füsun Sahin, Esra Yazar, Pinar Yildiz. *Department of Chest Diseases, Yedikule Chest Diseases and Thoracic Surgery Research and Training Hospital, Istanbul, Turkey*

Recent studies show that platelets (Plt.) have important roles in the immune system. It is known that MPV (mean platelet volume) and PDW (platelet distribution width) increase during platelet activation. Some research indicates their roles in coronary artery disease, thromboembolic disease and endotoxemia. The aim of our study, changes in platelet count and indices are investigated in pulmonary tuberculosis and pneumonia. Platelet count and indices were evaluated in 98 patients with active tuberculosis (mean age 38.78±15.42) and 35 patients with pneumonia (mean age 40.46±17.34) and 20 healthy control (mean age 36.20±11.62). Radiological extents of the diseases were assessed. In the active tuberculosis group values were significantly higher (Plt: 381683±125046; MPV: 8.57±1.39; PDW: 14.67±2.10; PCT (plateletcrit): 0.31±0.09) than pneumonia group (Plt: 283457±76101; MPV: 8.74±0.55; PDW: 13.74±1.34; PCT: 0.25±0.04) and healthy control group (Plt: 266150±55084; MPV: 8.57±0.42; PDW: 12.84±0.86; PCT: 0.24±0.03). In the pneumonia group values were significantly lower than active tuberculosis group; but only PDW values were significantly higher than healthy control group (p<0.05). Platelet count and PCT showed significant correlation with radiological extent of tuberculosis, while MPV and PDW correlations with radiological extent of tuberculosis were not significant. Plt, MPV, PDW and PCT correlations with radiological extent of pneumonia were not significant. These results emphasize that Plt, MPV, PDW and PCT change in tuberculosis. These changes may not reflect only disease activity and acute phase reaction. Plt and indices may be potential role in tuberculosis immunopathogenesis.

P4388

Urinary neopterin levels discriminate active from latent mycobacterium tuberculosis infection

Michael Eisenhut[1], Dougal Hargreaves[2], Anne Scott[3], David Housley[4], Andrew Walters[3], Rohinton Mulla[3], Parthipan Kanthapillai[5], James Ramsay[5], Oliver Eikenberg[6], Ralf Edelmann[6]. *[1]Department of Paediatrics, Luton&Dunstable Hospital NHS Foundation Trust, Luton, United Kingdom; [2]Institute of Child Health, University College London, United Kingdom; [3]Department of Medical Microbiology, Luton & Dunstable Hospital NHS Foundation Trust, Luton, United Kingdom; [4]Department of Clinical Biochemistry, Luton & Dunstable Hospital NHS Foundation Trust, Luton, United Kingdom; [5]Department of Respiratory Medicine, Luton & Dunstable Hospital NHS Foundation Trust, Luton, United Kingdom; [6]International Product Managment, IBL International GmbH, Hamburg, Germany*

Background: A biomarker to discriminate active from latent mycobacterium (m.) tuberculosis infection could help in exclusion of occult tuberculosis and avoid under treatment of those patients. Neopterin is released by macrophages in response to stimulation by interferon gamma.
Objective: To assess whether urinary levels of neopterin can discriminate between latent and active mycobacterium tuberculosis infection.
Methods: Urinary neopterin/creatinine ratio's were determined in patients with active and latent m. tuberculosis infection and controls without m. tuberculosis infection. Latent m. tuberculosis infection was defined as reactive interferon gamma release assay for m. tuberculosis in the absence of active disease.
Results: Seven patients with active tuberculosis, 27 patients with latent m. tuberculosis infection and seven controls were recruited. There was no difference in age or gender between groups. Urinary neopterin/creatinine ratio was higher in patients with active tuberculosis (412.8 micromol/mol, 95% CI 89.7 to 735.8) than patients with latent m tuberculosis infection (147.5 micromol/mol, 95% CI 114.0 to 180.8) and controls (122.2 micromol/mol, 95% CI 71.2 to 173.1) (p<.01). ROC curve analysis revealed an area under the curve of 0.81 (95% CI 0.62 to 0.99). A cut-off of 349 micromol/mol showed 100% diagnostic specificity in detection of active tuberculosis in people with m. tuberculosis infection.
Conclusions: Urinary neopterin/creatinine ratios are significantly higher in patients with active tuberculosis compared to patients with latent m. tuberculosis infection. These findings suggest that neopterin appears to be a suitable marker to reflect tuberculosis disease activity.

P4389

Delays in the diagnosis and treatment of tuberculosis in a south London hospital: The role of chest X-rays

Elena Karampini, Deepak S. Rao, Shahed Islam, Lucinda Dawson, Bassey Asuquo, Thomas C. Stokes. *Respiratory Medicine, Queen Elizabeth Hospital, London, United Kingdom*

Introduction: Early referral of patients with suspected tuberculosis (TB) has a significant impact to clinical outcomes, leading to shorter infectivity times and reduced morbidity and mortality rates.
Aim: To identify factors contributing to delays in the diagnosis of intra-thoracic (pulmonary, mediastinal and pleural) TB.
Methods: A retrospective case review of all patients who were diagnosed with intra-thoracic TB (January 2003 to January 2011) in Queen Elizabeth Hospital, Woolwich. Allowing for a chest X-ray (CXR) turn-around time of 3 weeks and a median period of 7 days between TB diagnosis and commencing treatment, we reviewed the full radiological history of all patients for whom the cut-off period of 28 days was exceeded. Delayed cases were divided into five groups, according to the reason for the delay:
1. Clinical diagnostic delay (unreported/misreported CXRs)
2. Delayed referral to specialist services
3. Pleural effusion (CXRs with effusions, subsequently proven to be tuberculous)
4. CXRs with concurrent pathologies
5. Lost to follow up

Results: 634 intra-thoracic TB notifications were made within the specified time period. 121 patients (19%) had at least one abnormal CXR taken 28 or more days prior to starting treatment (group 1: 38, group 2: 43, group 3: 16, group 4: 8, group 5: 16). The time delay between first abnormal CXR and starting treatment varied considerably (median: 69.5 days, range: 29–1020 days) and was greater in male (73%) and Asian patients (40%). 32 patients (26.4%) were sputum smear positive.
Conclusion: The diagnosis of RTB is delayed for a significant number of patients and appropriate measures should be taken in order to minimise such delays.

P4390

The role of bronchoalveolar lavage in suspected pulmonary tuberculosis with negative sputum

Cláudia Lares dos Santos[1], João Moura e Sá[4], Raquel Duarte[2,3,4,5]. *[1]Pulmonology Department, Hospital Centre of Coimbra, Coimbra, Portugal; [2]Pulmonology Diagnostic Centre, Pulmonology Diagnostic Centre, Vila Nova de Gaia, Portugal; [3]Reference Centre for Multidrug Resistant Tuberculosis, Pulmonology Diagnostic Centre, Vila Nova de Gaia, Portugal; [4]Pulmonology Department, Hospital Centre of Vila Nova de Gaia/Espinho, Vila Nova de Gaia, Portugal; [5]Medicine College, University of Porto, Porto, Portugal*

Introduction: Patients with suspected pulmonary tuberculosis and negative sputum samples constitute a problem that is not uncommon in clinical practice. Fiberoptic bronchoscopy is an alternative method of collecting respiratory samples that plays an important role in such cases.
Objectives: The aim of this study was to assess the yield of bronchoalveolar lavage in early diagnosis of negative sputum smear pulmonary tuberculosis and its value in obtaining other diagnoses.
Methods: The study was conducted for 27 months in a central hospital. Bronchoscopy was performed in patients after three consecutive negative sputum smears for acid-fast bacilli, in order to obtain bronchoalveolar lavage samples. Written informed consent was obtained.
Results: The overall diagnostic yield of fiberoptic bronchoscopy was 39% (39 out of 100) and included 18% of other diagnosis than tuberculosis with 3 cases of malignant disease. Through bronchoalveolar lavage smear and nucleic acid amplification test for M. tuberculosis an immediate diagnosis was obtained in 57.1%. Median time to positive culture was lower in bronchoalveolar lavage compared to sputum (p=0.001). The global resistance to antituberculosis drugs was 19%.
Conclusions: Bronchoalveolar lavage samples were helpful in the management of

smear negative pulmonary tuberculosis. The procedure had good diagnostic yield and contributed to the diagnosis of other diseases.

P4391

Diagnostic accuracy of sputum induction test compared with bronchoscopic results in patients with pulmonary tuberculosis

Yunseong Kim, Doosoo Jeon, Kwangha Lee, Kiuk Kim, Hyekyung Park, Minki Lee, Soonkew Park. *Internal Medicine, Pusan National University School of Medicine, Yangsan, Gyeongsangnam-do, Republic of Korea*

Rationale: Diagnosis of pulmonary tuberculosis is difficult in patient who do not produce sputum spontaneously, or who have AFB smear (-) sputum. Bronchoscopy is helpful in these patients, but in many cases cannot be readily available. We prospectively compared the diagnostic yield of sputum induction test with bronchoscopy.
Methods: From February 1 to July 31, 2010, we included the patients suspected active pulmonary tuberculosis, who could not produce sputum spontaneously, or who had a pair of AFB smear (-) sputum. They underwent sputum induction test and bronchoscopy. We calculated the sensitivity of AFB smear, culture for Mycobacterium tuberculosis, TB-PCR of each test, and evaluated the concordance rate by kappa test.
Results: Sensitivities of AFB smear were 36.1% in sputum induction test and 33.3% in bronchoscopy. Sensitivities of culture for Mycobacterium tuberculosis were 69.4% and 75.0%, and TB PCR were 52.8% and 58.3% in sputum induction and bronchoscopy, respectively. The results of AFB smear by sputum induction and bronchoscopy were concordant in 94% (63/67case, Kappa test=0.819). In culture for Mycobacterium tuberculosis, the results were concordant in 82% (54/67case, Kappa test=0.684) and the results of TB-PCR were concordant in 88% (59/67case, Kappa test=0.715).
Conclusions: In this study, sputum induction test had shown similar diagnostic value and sensitivity with bronchoscopy in the diagnosis of active pulmonary tuberculosis. In patients who are difficult in collecting sputum, or have AFB smear-negative sputum, sputum induction test can be an alternative approach to the diagnosis of active pulmonary tuberculosis.

P4392

Use of fibreoptic bronchoscopy in early diagnosis of sputum smear-negative pulmonary tuberculosis

Hoi-Yee Kwan[1], Susanna Lau[2], Kah-Lin Choo[1], Wai-Kei Lam[1], Chun-Man Wong[1], Chin-Wing Yu[1], Tze-Lun Wong[1], Wing-Yan Chu[1], Kwan-Keung Wong[1]. *[1]Department of Medicine, North District Hospital, The New Territories, Hong Kong; [2]Department of Microbiology, Queen Mary Hospital, Pokfulam, Hong Kong*

Background: Pulmonary tuberculosis (PTB) is a major health problem worldwide. Rapid diagnosis allows early treatment and infection control, which is hard to achieve among sputum smear-negative (SSN) subjects. Different bronchoscopic sampling techniques have been used but their roles remain unclear.
Objectives: To evaluate the value of fibreoptic bronchoscopy in the diagnosis of PTB among SSN patients in a regional hospital in Hong Kong.
Methods: Medical records of 22 patients, who have underwent bronchoscopy in the North District Hospital, HKSAR, in 2009, and were later diagnosed of having PTB, were reviewed. Results of their pulmonary specimens were recorded. The exclusive diagnostic test was identified.
Results: Bronchoalveolar lavage (BAL) was performed in all 22 cases. Positive acid-fast smear and culture were obtained in three (13.6%) and six cases (27.3%) respectively, providing the exclusive means of diagnosis for four cases (three from smear, one from culture). Molecular study from BAL was done in 14 cases, in which five cases were test positive (35.7%), two cases gave exclusive diagnosis. Transbronchial lung biopsy (TBLB) was performed in 19 cases. All were sent for histology, while six were sent for acid-fast bacilli (AFB) culture. Histology gave positive results in five cases (26.3%), which was the exclusive means of diagnosis for two. TBLB AFB smear was all negative, but three gave positive AFB culture. Among them, one provided exclusive diagnosis.
Conclusion: While sputum examination remains the cornerstone in diagnosing PTB, fibreoptic bronchoscopy plus various sampling techniques served as a useful adjunct to optimize the diagnostic yield, especially among those SSN cases.

P4393

Rapid molecular detection of rifampicin and isoniazid resistance and identification of mutations in resistant genes of multi-drug resistant tuberculosis (MDR-TB) patients

Gulsum Ari[1], Can Bicmen[2], Onur Karaman[1], M. Sevket Dereli[1], Serir Aktogu Ozkan[1]. *[1]Chest Diseases, Dr. Suat Seren Chest Diseases and Surgery Training and Research Hospital, Izmir, Turkey; [2]Microbiology, Dr. Suat Seren Chest Diseases and Surgery Training and Research Hospital, Izmir, Turkey*

Recently identification of mutations responsible for drug resistance by molecular methods are used to detect antimycobacterial resistance in MDR-TB patients and to overcome difficulties of treatment planning in some TB patients. In our study 29 patients were evaluated- 16 patients who were treated with minor therapy or with suspection of MDR-TB and 13 patients with treatment failure, relapsing and returning after defaulting, who were taking retreatment regime. Mutations in rpoB, katG and inhA gene zones specific for rifampicin and isoniazid were investigated with molecular methods in 14 patients from direct smear positive samples and in 15 patients from positive culture. AFB stain, culture and drug susceptibility testing with BACTEC 460 were also done for all samples. 28 samples (22 MDR-TB, 5 susceptible to four drugs, 1 culture negative) were identified as M. tuberculosis complex, 1 patient was detected as M. intracellulare. 27 Patients who were culture positive for MTB were positive with molecular methods, 1 patient who was determined as M. intracellulare was found negative. As compared with drug susceptibility testing, rifampicin resistance was present in all 22 samples (%100) who were determined as MDR-TB and isoniazid resistance was detected in 21 (%95.5) patients. In 5 patients who were susceptible to four drugs, no mutation was found. In 3 patients with HR resistance, cure was achieved with retreatment regime.
Conclusion: The detection of HR resistance with molecular methods is a guide to diagnosis of MDR-TB patients and shortens the time to starting of MDR-TB treatment.

P4394

Real time polymerase chain reaction (RT-PCR) based rapid detection of multi-drug resistant (MDR) mycobacterium tuberculosis (MTB)

T.K. Jayalakshmi, Mansi Rathore, Girish Pai, Dattatray Joshi. *Dept of TB, Jaiclinic, Mumbai, Maharashtra, India Biotechnology Research, Mahatma Gandhi Mission Hospital, Navi Mumbai, Kamothe, Maharashtra, India*

Objective: Rapid confirmation of diagnosis of MTB and MDR-MTB in clinical samples by RT PCR and Melting Curve Analysis (MCA).
Introduction: TB is one of the leading causes of mortality in world. An extremely worrisome aspect of MTB is the recent rise in MDR- MTB cases in several countries. The culture based diagnostic procedures take weeks to detect TB and its drug resistant variants. In developing countries this delay could compromise efforts to interrupt TB transmission. There is, therefore, intense interest to develop rapid and precise molecular diagnostic methods.
Methods: DNA was extracted from sputum or body fluids of about 100 patients suspected of TB. MTB diagnosis was confirmed by RT PCR amplification and subsequent melting curve analysis, using IS6110 specific primers on Lightcycler480 (Roche Inc). For MDR MTB diagnosis hydrolysis probes were used to genotype mutations in rpoB and katG genes, associated with resistance to rifampin (RIF) and Isoniazide (INH) respectively.The data obtained from culture and molecular methods was compared.
Results: MTB diagnosis was confirmed by RTPCR with 100% sensitivity on sybrgreen format. For detection of MDR, complete agreement between diagnoses from RT-PCR and Culture was observed in 38 cases out of 40 cases. 2 false positive for RIF resistance were was observed. No false positive or false negative cases were observed for resistance to INH in this study.
Conclusions: RT PCR based assay with MCA has considerable promise for confirmation of diagnosis of MTB and MDR MTB. Development of more specific probes can further improve its diagnostic potential.

P4395

Effectiveness of TB diagnostics with microbiological methods in TB service and general health care institutions

Larisa Rusakova, Punga Victor, Gergert Vladislav. *Epidemiology, CTRI RAMS, Moscow, Russian Federation Immunology, CTRI RAMS, Moscow, Russian Federation*

Objective: To evaluate the effectiveness of TB diagnostics with microbiological methods in 41 574 newly detected TB patients.
Materials and methods: The effectiveness of TB diagnostics with microbiological methods in newly detected TB patients in 2007-2009 in clinicodiagnostic laboratories (CDL) of general health care (GHC) and TB service institutions was analyzed according to official applied-research data and the results obtained by the institute staff during supervisory visits. Analysis of TB diagnostics was assessed according to the indicators established by the Federal Target Programme (FTP), World Bank and GFATM projects.
Results: In 2007-2009 the proportion of SS+ among TB suspect patients examined by microscopy in CDL of GHC institutions increased by 33,3%. The indicator (1-5%) in 2009 was achieved in 7 out of 15 Russian regions. The proportion of newly detected pulmonary TB patients, SS+ by microscopy, among all registered SS+ by microscopy new cases of pulmonary TB has increased by 11,6% (2007 - 24,3%, 2008 - 28, 3%, 2009 - 27,5%). The proportion of newly detected patients, SS+ by microscopy, examined in CDL of TB service institutions, increased by 1,0% (48,8% and 49,3% accordingly).
Conclusion: In general, thanks to funding from different sources (FTP, World bank and GFATM) during 2005 - 2009 all doctors and laboratory technicians of TB service and GHC institutions were trained to detect TB with microbiological methods, the laboratory equipment and supplies were received and put to use and that resulted in improving performance quality of laboratory service.

P4396

Laboratory diagnostics of pulmonary mycobacteriosis

Larysa Surkova, Aksana Zalutskaya, Evgeni Sahalchyk. *Laboratory, National Research and Practical Centre for Pulmonology and TB, Minsk, Belarus*

Increase in risk groups on mycobacteriosis (AIDS and other immunosupressed patients), difficulties in differential diagnostics with TB, differences in mycobacteriosis incidence in different countries demand studying regional features of nontuberculous mycobacteria (NTM) occurrence and improving mycobacteriosis diagnostics.

The aim of the study was definition of occurrence and clinical value of NTM and improving mycobacteriosis diagnostics.

In 2009, 45 NTM cultures were isolated from 23 patients with TB or TB suspicion in centre using solid media and BACTEC MGIT 960. The proportion of NTM among all mycobacterial cultures was 0.9%. NTM identification was carried out using biochemical and cultural tests and molecular method Genotype Mycobacterium CM (Hain Lifescience, Germany).

60.9% NTM strains isolated from 14 patients were fast-growing (M. abscessus, M. fortuitum, M. chelonae, M. peregrinum, M. smegmatis, M. phlei) and 39.1% strains isolated from 9 patients were slowly growing (M. avium complex, M. kansasii, M. gordonae, M. terrae). 73.9% NTM strains isolated from 17 patients were clinically relevant; NTM strains isolated from 6 patients hadn't clinical relevance. NTM cultures were isolated many times from 10 patients, once from 13 patients. Mixed cultures (M. tuberculosis and NTM) were isolated from 5 patients. This fact can be evidence of TB superinfection in patients with mycobacteriosis during hospital stay.

Mycobacteriosis can be diagnosed in patients with pulmonary inflammatory process progressing in spite of antituberculous therapy in case of NTM culture isolation in 3 samples and more. Laboratory diagnostics of pulmonary mycobacteriosis should include a complex of microbiological and molecular methods.

P4397

Diagnostic culture confirmation and bacteriological evidence of cure in English adult TB cases: Can we do better?

Abbey Leahy[1], Martin Hetzel[1], Onn Kon[2], Marc Lipman[3], Ibrahim Abubakar[4]. *[1]Respiratory Medicine, Bristol Royal Infirmary, University Hospitals Bristol NHS Foundation Trust, Bristol, United Kingdom; [2]Respiratory Medicine, St, Mary's Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom; [3]Respiratory Medicine, Royal Free Hospital Hampstead NHS Trust, London, United Kingdom; [4]Infectious Disease Epidemiology, National Centre for Infections, Health Protection Agency, Colindale, London, United Kingdom*

Background: WHO and European guidelines recommend high levels of culture confirmation of pulmonary tuberculosis (TB) cases, and that clinicians report evidence of bacteriological cure. To date these criteria are not used in the UK.

Objectives: 1.To determine why not all cases of notified pulmonary TB have microbiological confirmation, and to identify factors required to improve the proportion of cases confirmed by culture. 2. To investigate the feasibility of obtaining bacteriological cure in culture confirmed cases.

Methods: Records for adults diagnosed with pulmonary TB and notified in 2009 from 3 hospitals in England (Bristol Royal Infirmary, St Mary's and Royal Free) were reviewed. A standard tool collected clinical and demographic data.

Results: 123 cases were identified (85% confirmed HIV negative). 95% of subjects had sputum or lung fluid samples sent for smear and culture. 58% of subjects had smear positive disease. Culture was positive in 79% cases. 5% had no cultures performed - mainly because non-TB specialists requested samples. At treatment completion, 16% of subjects were documented as microbiologically cured and 83%not tested. The main reasons were absence of symptoms and radiological resolution.

Conclusion: To improve bacterial confirmation at diagnosis, current culture techniques need enhancing. An awareness of needing specific mycobacterial samples amongst non-TB specialists may help. Given that documentation of microbiological cure is rarely performed, optimising simple sputum collection may be the only option as it is unlikely that induced sputum or bronchoscopy in asymptomatic patients at treatment end is acceptable, and unlikely to be cost effective.