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**ABSTRACTS**


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**Reducing the number of sputum samples examined and thresholds for positivity**

M. Bonnet, A. Ramsay, L. Gagnidze, N. Githui, P.J. Guerin and F. Caraine. *Int J Tuberc Lung Dis* 2007; **11(9)**: 953-958.

The objective was to evaluate the impact on tuberculosis case detection and laboratory workload of reducing the number of sputum smears examined and thresholds for diagnosing positive cases. In this prospective study, three Ziehl-Neelsen stained sputum smears from consecutive pulmonary TB suspects were examined blind. The standard approach (A),  $\geq 2$  positive smears out of 3, using a cut-off of 10 acid-fast bacilli (AFB)/100 high-power fields (HPF), was compared with approaches B,  $\geq 2$  positive smears ( $\geq 4$  AFB/100 HPF) out of three, one of which is  $\geq 10$  AFB/100 HPF; C,  $\geq 2$  positive smears ( $\geq 4$  AFB/100 HPF) out of 3; D,  $\geq 1$  positive smear ( $\geq 10$  AFB/100 HPF) out of 2; and E,  $\geq 1$  positive smear ( $\geq 4$  AFB/100 HPF) out of two. The microscopy gold standard was detection of at least one positive smear ( $\geq 4$  AFB/100 HPF) out of three. Among 644 TB suspects, the alternative approaches detected from 114 (17.7%) (approach B) to 123 cases (19.1%) (approach E) compared to 105 cases (16.3%) for approach A ( $P < 0.005$ ). Sensitivity ranged between 82.0% (105/128) for A and 96.1% (123/128) for E. The single positive smear approaches reduced the number of smears by 36% compared to approach A. Reducing the number of specimens and the positivity threshold to define a positive case increased the sensitivity of microscopy and reduced laboratory workload.

**Ethambutol in paediatric tuberculosis: aspects of ethambutol serum concentration, efficacy and toxicity in children**

S. Thee, A. Detjen, D. Quarcoo, U. Wahn and K. Magdorf. *Int J Tuberc Lung Dis* 2007; **11(9)**: 965-971.

Ethambutol (EMB) is used as a fourth drug in paediatric anti-tuberculosis treatment. In current

recommendations the dosage of EMB is calculated per kg body weight. The objective was to present two studies investigating an appropriate 3MB dosage in children, and observational data on its toxicity and efficacy. EMB serum levels in children of different age groups were determined after single oral administration of EMB alone as well as after EMB combined with rifampicin, and optimal dosages were established. The efficacy and toxicity of these EMB dosages were examined retrospectively. EMB serum levels were lower than those expected in adults receiving a similar oral dose, due to different pharmacokinetics and pharmacodynamics in childhood. Thereafter, children were treated with EMB doses calculated by body surface ( $867/\text{m}^2$ ). Ocular toxicity occurred in 0.7% of cases and relapse in 0.8%. Current recommended EMB dosages in childhood tuberculosis lead to subtherapeutic serum levels. It appears to be more valid to calculate the EMB dosage on the basis of body surface rather than body weight, leading to higher dosages especially in younger children. With these dosages, therapeutic serum levels are reached in all age groups, leading to a high efficacy of anti-tuberculosis treatment without increased ocular toxicity.

**Micro-nutrient supplements and mortality of HIV-infected adults with pulmonary TB: a controlled clinical trial**

R.D. Semba, J. Kumwenda, E. Zijlstra, M.O. Ricks, M. van Lettow, C. Whalen, T.D. Clark, L. Jorgensen, J. Kohler, N. Kumwenda, T.E. Taha and A.D. Harries. *Int J Tuberc Lung Dis* 2007; **11(8)**: 854-859.

The aim of the trial was to determine whether daily micro-nutrient supplementation reduces the mortality of human immunodeficiency virus (HIV) infected adults with pulmonary tuberculosis (TB). It was a randomized, controlled clinical trial of micronutrient supplementation for HIV-positive and HIV-negative adults with pulmonary TB. Participants were enrolled at the commencement of chemotherapy for sputum smear-positive pulmonary TB and followed up for 24 months. A

total of 829 HIV-positive and 573 HIV-negative adults were enrolled. During follow-up, 328 HIV-positive and 17 HIV-negative participants died. The proportion of HIV-positive participants, who died in the micronutrient and placebo groups was 38.7% and 40.4%, respectively ( $P = 0.49$ ). Micronutrient supplementation did not reduce mortality (hazard ratio [HR] 0.93, 95% CI 0.75-1.15) among HIV-positive adults. Micro-nutrient supplementation at the doses used in this study does not reduce mortality in HIV-positive adults with pulmonary TB.

**Evaluation of post-treatment health-related quality of life (HRQoL) among tuberculosis patients**

M. Muniyandi, R. Rajeswari, R. Balasubramanian, C. Nirupa, P.G. Gopi, K. Jaggarajamma, F. Sheela and P.R. Narayanan. *Int J Tuberc Lung Dis* 2007; **11(8)**: 887-892.

Health-related quality of life (HRQoL) measures the impact of a disease by assessing the health status of patients. The objective was to assess the HRQoL of tuberculosis (TB) patients one year after treatment completion. Patients registered under the TB control programme from July 2002 to June 2003 in a TB Unit in South India were interviewed one year after successful completion of treatment. Data on HRQoL were collected using the SF-36 questionnaire, which covers physical, mental and social well-being components. Data on economic well-being were also collected. Scores were given for all domains. Of 436 TB patients interviewed, the mean scores for social, physical, mental and economic well-being were respectively 84, 74, 68 and 62 on a scale of 100. The well-being scores were significantly related to age, sex, education, employment and persistent symptoms. There was a significant association between economic and social well-being. This study suggests that the HRQoL of TB patients one year after successful completion of treatment under the TB control programme was normal for most of the domains studied and was associated with age, literacy and employment, income, smoking, alcoholism and persistence of symptoms.

**Is it valuable to examine more than one sputum smear per patient for the diagnosis of pulmonary tuberculosis?**

Aydan Oszkutuk, Gulfem Terek, Huseyin Coban and Nuran Esen. *Jpn J Infect Dis* 2007; **60**: 73-75.

The simplest, cheapest, and fastest diagnostic method for tuberculosis is the detection of acid-fast bacilli by microscopy. The algorithm advised for the diagnosis of TB recommends examination of three consecutive sputum specimens from TB suspects for the presence of AFB. In the present study, we evaluated the contribution of each specimen to the final detection of TB suspect patients with culture-proven disease. The collection and analysis of retrospective data on patients with culture-proven pulmonary TB, from June 2002 to August 2006, at Dokuz Eylul University Hospital, Turkey, have enabled us to assess the value of examining two sputum specimens in diagnosing this disease. AFB were detected from one or more sputum specimens with direct microscopy in 42% of the cases. An analysis of results of smear examination showed that 97% of AFB were detected from the first specimen and only 3 % were obtained from the second smear. The third specimen did not have any additional diagnostic value for the detection of AFB by microscopy. As a conclusion the present study shows that examining two sputum smears is sufficient for the early detection of AFB in our laboratory.

**Comparison of the tuberculin skin test and the quantiferon test for latent *mycobacterium tuberculosis* infections in health care workers.**

D. Ozdemir, A.N. Annakkaya, G. Tarhen, I. Sencan, S. Cesur, O. Balbay and E. Guclu. *Jpn J Infect Dis* 2007; **60**: 102-105.

Aim of this study was to compare the efficacy of the tuberculin skin test (TST) and the quantiferon test (QFT) for detecting latent tuberculosis infection (LTBI) in health care workers (HCWs). Seventy-six participants who were working in Duzce University Hospital, where tuberculosis patients were being treated, were included in the study. TST was performed according to the Mantoux technique. QFT was performed in accordance with the manufacturer's instructions. A

positive TST result was defined as an induration diameter of  $\geq 15$  mm. TSTs were positive in 41 of 76 participants (53.9%) and QFT was positive in 65 of 76 participants (85.5%). There was a significant difference between the numbers of QFT- positive and TST-positive cases ( $P = 0.02$ ). When the induration diameter of TST was  $\geq 20$  mm, QFT positivity was 100%. Multivariate analysis revealed that there was a significant correlation between the percentage of patients with QFT positivity and the induration diameter of TST ( $P = 0.009$ ). QFT thus seems to be more effective for LTBI diagnosis than TST. However, large-scale trials including quantitative measurement of QFT in subgroups taking into account the division where HCWs are employed and the different results of TST might clarify the usefulness of QFT in LTBI diagnosis

#### **Correlation of sputum culture with serology against *Mycoplasma pneumoniae* in patients with bronchial asthma**

Rameshchandra Sahoo, K. Vishak Acharya, M. Shalini Shenoy, R. Anand and Rama Keshava Reddy. *Indian J of Chest Dis & Allied Sci* 2007; **49(4)**:209-212.

*Mycoplasma pneumoniae* is implicated in acute exacerbations of bronchial asthma and is also a factor in chronicity of asthma. We attempted to isolate *M. pneumoniae* in sputum specimen samples by culture technique and also by serological estimation of immunoglobulin (IgM, IgG) antibodies against *M. pneumoniae* by enzyme linked immunosorbent assay (ELISA) in 100 patients with bronchial asthma and 50 subjects without asthma who served as controls. *Mycoplasma pneumoniae* was isolated by sputum culture in 17% of patients with asthma and in none of the control subjects. Immunoglobulin (IgG / IgM) antibodies against *M. pneumoniae* were found in 37% of patients with asthma and 6% of control subjects. On correlating culture with serology, it was found that 15% patients were both culture and seropositive, 2% patients were culture positive but seronegative, 22% patients were culture negative but seropositive while 61 % patients were both culture and seronegatives. The sensitivity and specificity of culture were 73.5% and 88.2%, respectively. For the serological method, sensitivity and specificity were 88.2% and 73.5%, respectively.

Use of a combination of sputum culture and serological methods are reliable investigational tools for the diagnosis of *M. pneumoniae* infection. The significant association observed between *M. pneumoniae* and bronchial asthma justifies the inclusion of antibiotics, such as the macrolides, in treating these patients.

#### **Interleukin-12B and interleukin-10 gene Polymorphisms in pulmonary tuberculosis**

S. Prabhu Anand, P. Selvaraj, M.S. Jawahar, A.R.Adhilakshmi and P.R. Narayanan. *Indian J MedRes* 2007; **126**: 135-138.

Cytokines play an important role in anti-tuberculosis immune response. Skewing of immunity from protective to pathogenic may involve a shift in Th1- Th2 paradigm. Cytokine gene polymorphism is known to be associated with functional differences in cytokine regulation and altered clinical performance in a variety of diseases. The aim of this study was to know whether Interleukin-12B 3' UTR (TaqI) (A/C) and Interleukin-10 (-1082 G/A) gene polymorphisms were associated with susceptibility to pulmonary tuberculosis. IL -10 (-1,082 G/A) and IL-12B gene polymorphisms were studied in 132 pulmonary TB (PTB) patients and 143 normal healthy subjects (NRS), using DNA based polymerase chain reaction (PCR) with sequence specific primers and restriction digestion. The allelic as well as genotypic frequencies of Interleukin -10 (-1082) and Interleukin -12B (3'UTR Taq I) did not differ significantly between the patients and controls. Our findings suggested that IL -10 (-1082 G/A) and IL -12B 3'UTR (Taq I) (A/C) gene polymorphisms were not associated either with susceptibility or resistance to pulmonary tuberculosis in the south Indian population.

#### **Association of mycobacteria with Eales' disease**

K.L. Therese, P. Deepa, J. Therese, R. Bagyalakshmi, J. Biswas and R.N. Madhavan. *Indian J Med Res* 2007; **126**: 56-62.

Eales' disease is an idiopathic disease resulting in retinal neovascularization, recurrent haemorrhages, with or without retinal detachment predominantly affecting healthy young males (97.6%) in the Indian subcontinent. In spite of several studies, the aetiology of Eales' disease is not clear. The isolation

of *Mycobacterium fortuitum* from the aqueous humour of a patient with classical Eales' disease, led us to hypothesize that rapid growing non-tuberculous mycobacteria (RGNTM), particularly *M. fortuitum* and *M. chelonae* could be associated with Eales' disease. We, therefore, undertook this study to detect DNA of these RGNTM and also of *M. tuberculosis* in vitreous fluids (VFs) from patients with Eales' disease and non-Eales' disease. We developed and optimized seminested polymerase chain reactions (SnPCRs) to detect DNAs of *M. fortuitum* and *M. chelonae* on archival ERMs (33) and VFs (19) of Eales' and control patients along with conventional mycobacteriological investigations. In this retrospective study, 70 per cent ERM samples were positive for one or more *Mycobacterium spp.* tested by snPCR. *M. fortuitum* and *M. chelonae* were isolated from two VFs, which were also positive by snPCR in the prospective study. Statistical evaluation of the results of both retrospective and prospective investigations showed a statistically significant association of *Mycobacterium spp.* with Eales' disease. The results of the present study suggested the involvement of *mycobacterium spp.* in the aetiopathogenesis of Eales' disease. Further studies on a larger sample will be required to confirm these findings.

**Molecular typing of *Mycobacterium tuberculosis* isolates from different parts of India based on IS6110 element polymorphism using RFLP analysis**

D.S. Chauhan, V.D. Shanna, Deepti Parashar, Aradhana Chauhan, D. Singh, H.B. Singh, R. Das, B.M. Aggarwal, B. Malhotra, Amita Jain, Meera Shanna, V.K. Kataria, J.K. Aggarwal, Mohamad Ifanif, Aruna Shahani and V.M. Katoch. *Indian J Med Res* 2007; **125**: 577-581.

IS 6110 based typing remains the internationally accepted standard and continues to provide new insights into the epidemiology of *Mycobacterium tuberculosis*. The aim of the study was to characterize *M tuberculosis* isolates obtained from different parts of India based on IS 6110 element polymorphism using restriction fragment length polymorphism (RFLP) analysis. RFLP was analyzed among 308 isolates of *M. tuberculosis* deposited in the Mycobacterial Repository Centre, Agra, from

different parts of India. DNAs isolated from these strains were restricted with Pvu II, transferred on to nylon membrane and hybridized with a PCR amplified DIG-labelled 245 bp IS6110 probe. Based on the copy number, *M. tuberculosis* isolates were classified into four groups, (i) lacking IS6110 element (ii) low copy number (1-2); (iii) intermediate copy number (3-5); and (iv) high copy number (6-19). Copy number higher than 19, however, was not observed in any of the isolates studied. At the national level, 56 per cent of the isolates showed high copy number of IS6110, 13 per cent showed intermediate copy number, 20 per cent showed low copy number, whereas 11 per cent isolates lacked IS 6110 element. At the regional level, there was not much difference in the RFLP profiles of isolates (IS 6110 copy numbers/patterns) from different parts of the country. IS 6110 DNA based finger printing could be a potentially useful tool for investigating the epidemiology of tuberculosis in India.

**Urine levels of rifampicin and isoniazid in asymptomatic HIV-positive individuals.**

Geetha Ramachandran, A.K. Hemanth Kumar, K. Sarala, C. Padmapriyadarsini, S. Anitha, C.B. Tharani, V Kumaraswami and Soumya Swaminathan. *Indian J Med Res* 2007; **125**: 577-581.

AIDS and its associated gastro-intestinal complications may impair the absorption of anti-tuberculosis (TB) drugs. Impaired absorption of anti-TB drugs could lead to low drug exposure, which might contribute to acquired drug resistance and reduced effectiveness of anti-TB treatment. The aim of this study was to obtain information on the status of absorption of rifampicin (RMP) and isoniazid (INH) in asymptomatic HIV-positive individuals, who are less immuno-compromised. The D-xylose absorption test was also carried out to assess the absorptive capacity of intestine. The absorption of RMP, INH and D-xylose was studied in 15 asymptomatic HIV-positive individuals with CD4 cell counts >350 cells/mm<sup>3</sup> and 16 healthy volunteers, after oral administration of single doses of RMP (450 mg), INH (300 mg) and D-xylose (5 g). Urine was collected up to 8 h after drug administration. Percentage dose of the drugs and their metabolites and D-xylose excreted in urine were calculated. A

significant reduction in the urinary excretion of INH and D-xylose in HIV-positive persons compared to healthy volunteers was observed. The per cent dose of RMP and its metabolite, desacetyl RMP was also lower in HIV-positive persons compared to healthy volunteers, but this difference was not statistically

significant. Decreased urinary excretion of D-xylose and INH are suggestive of intestinal malabsorption in HIV-positive individuals. HIV infection could cause malabsorption of anti-TB drugs even at an early stage of the disease. The clinical implications of these findings need to be confirmed in larger studies.

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