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754 KIVIHYA-NDUGGA, L.; CLEEFF, M. VAN; NYAMWAYA, J.; MIHESO, B.; NDALO, E.; ODHIAMBO, J.; KLATSER, P. **Determinants of tuberculosis diagnosis and the role of counselling.** *East African Medical Journal* (2007) **84** (2) 77-82 Nairobi, Kenya; Kenya Medical Association [En, 13 ref.] Center for Respiratory Diseases Research, Kenya Medical Research Institute, P.O. Box 47855-00100, Nairobi, Kenya.

Objective: To study patient determinants that may affect completion of the diagnostic process in tuberculosis control, highlighting the role of counselling. Design: Cross-sectional study. Subjects: TB patients. Setting: Rhodes Chest Clinic, Nairobi, City Council. Results: Ninety five percent of the suspects delivered three sputum samples but only 27% consented to a HIV test; several determinants for none consenting were mentioned. On average US$ 2.27 was spent for one clinic visit and US$ 8.62 for following the entire diagnostic process. Cost factors included transport, loss of income and food. Conclusion: Individual pre-test counselling seems important for obtaining three sputum specimens. It takes time and for settings with a large number of suspects, alternative' methods maybe required. To obtain consensus for a HIV test in a TB clinic is complicated. Costs spent on transport and loss in income are important determinants and may contribute to poor patient adherence to the diagnostic process.

755 CHAUTY, A; ARDANT, M. F.; ADEYE, A; EUVERTE. H.; GUÉDÉNON. A.; JOHNSON, C.; AUBRY, J.; NUERMBERGER. E.; GROSSET, J. **Promising clinical efficacy of streptomycin-rifampin combination for treatment of Buruli ulcer (*Mycobacterium ulceralls* disease).** *Antimicrobial Agents and Chemotherapy* (2007) **51** (11) 4029-4035 Washington, USA; American Society for Microbiology (ASM) [En, 25 ref.] Céntre de Depistage et de Traitement de l'ulcère de Buruli, Pobè, Benin. Email: jgrosse4@jhmi.edu

According to recommendations of the 6th WHO Advisory Committee on Buruli ulcer, directly observed treatment with the combination of rifampin and streptomycin, administered daily for 8 weeks, was recommended to 310 patients diagnosed with Buruli ulcer in Pobè, Bénin. Among the 224 (72.%) eligible patients for whom treatment was initiated, 215 (96%) were categorized as treatment successes, and 9, including 1 death and 8 losses to follow-up, were treatment failures. Of the 215 successfully treated patients, 102 (47%) were treated exclusively with antibiotics and 113 (53%) were treated with antibiotics plus surgical excision and skin grafting. The size of lesions at treatment initiation was the major factor associated with surgical intervention: 73% of patients with lesions of >15 cm in diameter underwent surgery, whereas only 17% of patients with lesions of <5 cm had surgery. No patient

discontinued therapy for side effects from the antibiotic treatment. One year after stopping treatment, 208 of the 215 patients were actively retrieved to assess the long-term therapeutic results: 3 (1.44%) of the 208 retrieved patients had recurrence of *Mycobacterium ulcerans* disease, 2 among the 107 patients treated only with antibiotics and 1 among the 108 patients treated with antibiotics plus surgery. We conclude that the WHO-recommended streptomycin-rifampin combination is highly efficacious for treating *M. ulcerans* disease. Chemotherapy alone was successful in achieving cure in 47% of cases and was particularly effective against ulcers of less than 5 cm in diameter.

756 SCHIERLOH. P.; YOKOBORI, N.; ALEMAN. M.; LANDONI, V.; GEFFNER. L.; MUSELLA. R. M.; CASTAGNINO. J.; BALDINI. M.; ABBATE. E.; BARRERA. S. S. DE LA; SASIAIN. M. C. ***Mycobacterium tuberculosis*-induced gamma interferon production by natural killer cells requires cross talk with antigen-presenting cells involving toll-like receptors 2 and 4 and the mannose receptor in tuberculous pleurisy.** *Infection and Immunity* (2007) **75** (11) 5325-5337 Washington, USA; American Society for Microbiology (ASM) [En, 54 ref.] Departamento de Inmunología, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Pacheco de Melo 3081, 1425 Buenos Aires, Argentina. Email: msasiain@hematologia.anm.edu.ar

Tuberculous pleurisy allows the study of human cells at the site of active *Mycobacterium tuberculosis* infection. In this study, we found that among pleural fluid (PF) lymphocytes, natural killer (NK) cells are a major source of early gamma interferon (IFN-) upon *M. tuberculosis* stimulation, leading us to investigate the mechanisms and molecules involved in this process. We show that the whole bacterium is the best inducer of IFN- , although a highmolecular-weight fraction of culture filtrate proteins from *M. tuberculosis* H37Rv and the whole-cell lysate also induce its expression. The man nose receptor seems to mediate the inhibitory effect of mannosylated lipoarabinomannan, and Toll-like receptor 2 and 4 agonists activate NK cells but do not induce IFN- like *M. tuberculosis* does. Antigen-presenting cells (APC) and NK cells bind *M. tuberculosis*, and although interleukin-12 is required, it is not sufficient to induce IFN- expression, indicating that NK cell-APC contact takes place. Indeed, major histocompatibility complex class I, adhesion, and costimulatory molecules as well as NK receptors regulate IFN- induction. The signaling pathway is par-tially inhibited by dexamethasone and sensitive to Ca^{2+} flux and cyclosporine. Inhibition of p38 and extracellular-regulated kinase mitogen-activated protein kinase pathways reduces the number of IFN- $^{+}$ NK cells. Phosphorylated p38 (p-p38) is detected in ex vivo PF-NK cells, and *M. tuberculosis* triggers p-p38 in PF-NK cells at the same time that binding between NK and *M. tuberculosis* reaches its maximum value. Thus, interplay between *M. tuberculosis* and NK cells/APC triggering IFN- would be expected to play a beneficial role in tuberculous pleurisy by helping to maintain a type 1 profile.

757 SHARMA, N.; MALHOTRA, R.; TANEJA, D. K.; SAHA, R; INGLE, G. K. **Awareness and perception about tuberculosis in the general population of Delhi.** *Asia-Pacific Journal of Public Health* (2007) **19** (2) 10-15 Kuala Lumpur, Malaysia; Asia-Pacific Academic Consortium for Public Health [En, 8 ref.] Department of Community Medicine, Maulana Azad Medical College, H-41, TypeV, Nivedita Kunj, R K Puram Sector-l0, New Delhi - 110 022, India. Email: drnandinil@gmail.com

The present study was conducted to assess awareness and perception toward tuberculosis (TB) among the general population of Delhi, India. A total of 1008 adults, selected by multistage stratified systematic sampling, were interviewed using a pre-tested proforma. The majority had heard about TB (99.1%) and most (89.2%) perceived it to be an infectious disease. The correct mode of transmission, i.e., airborne (coughing/sneezing), was known to 71.8% of study subjects. The majority (90.1%) knew cough as a symptom. Nearly all (98.2%) perceived TB to be a preventable disease, citing the treatment of patients as the mainstay of preventing spread of the disease. However, responses such as separation of utensils or hospitalization of the patient to prevent the spread of the disease indicate persistence of stigma and discrimination in a small proportion of the population. There is a need to widen the scope and intensify the information and education being provided to the population based on gaps identified.

758 VREE, M.; HUONG, N. T.; DUONG, B. D.; CO, N. V.; SY, D. N.; COBELENS, F. G.; BORGDORFF, M. W. **High mortality during tuberculosis treatment does not indicate long diagnostic delays in Vietnam: a cohort study.** *BMC Public Health* (2007) **7** (210) (16 August 2007) London, UK; BioMed Central Ltd [En, 32 ref.] Research unit, KNCV Tuberculosis Foundation, PO Box 146, 2501 CC, The Hague, Netherlands. Email: vreem@kncvtbc.nl. nthuong139@gmail.com, bdduong06@gmail.com, sonlinh_2001@yahoo.com, ngocsyvienlao@yahoo.com, cobelensf@kncvtbc.nl, borgdorff@kncvtbc.nl

Background: Delay in tuberculosis diagnosis and treatment initiation may increase disease severity and mortality. In evaluations of tuberculosis control programmes high fatality rates during tuberculosis treatment, are used as an indicator of long delays in low HIV-prevalence settings. However, data for this presumed association between delay and fatality are lacking. We assessed the association between diagnostic delay and mortality of new smear- positive pulmonary tuberculosis patients in Vietnam. Methods: Follow-up of a patient cohort included in a survey of diagnostic delay in 70 randomly selected districts. Data on diagnosis and treatment were extracted from routine registers. Patients who had died during the course of treatment were compared to those with reported cure, completed treatment or failure (survivors). Results: Complete data were available for 1881/2093 (89.9%) patients, of whom 82 (4.4%) had died. Fatality was 4.5% for patients with 4 weeks delay, 5.0% for 5- 8 weeks delay (aOR 1.11, 95%CI 0.67-1.84) and 3.2% for >9 weeks delay (aOR 0.69, 95%CI 0.37-1.30). Fatality tended to decline with increasing delay but this was not significant. Fatality was not associated with median diagnostic delay at district level (Spearman's rho=-0.08, P=0.5). Conclusion: Diagnostic delay is not associated with treatment mortality in Vietnam at individual nor district level, suggesting that high case fatality should not be used as an indicator of long diagnostic delay in national tuberculosis programmes.

759 BARAL, S. C.; KARKI, D. K.; NEWELL, J. N. **Causes of stigma and discrimination associated with tuberculosis in Nepal: a qualitative study.** *BMC Public Health* (2007) **7** (211) (16 August 2007) London, UK; BioMed Central Ltd [En, 36 ref.] Nuffield Centre for International Health and Development, Leeds Institute for Health Sciences, University of Leeds, Charles Thackrah Building, 101 Clarendon Road, Leeds, LS2 9LJ, UK. Email: sushilb@mos.com.np, dekarki@wlink.com.np, j.n.newell@leeds.ac.uk

Background: Tuberculosis (TB) is a major cause of death. The condition is highly stigmatised, with considerable discrimination towards sufferers. Although there have been several studies assessing the extent of such discrimination, there is little published (research explicitly investigating the causes of the stigma and discrimination associated with TB. The objectives of our research were therefore to take the first steps towards determining the causes of discrimination associated with TB. Methods: Data collection was performed in Kathmandu, Nepal. Thirty four in-depth interviews were performed with TB patients, family members of patients, and members of the community. Results: Causes of self-discrimination identified included fear of transmitting TB, and avoiding gossip and potential discrimination. Causes of discrimination by members of the general public included: fear of a perceived risk of infection; perceived links between TB and other causes of discrimination, particularly poverty and low caste; perceived links between TB and disreputable behaviour; and perceptions that TB was a divine punishment. Furthermore, some patients felt they were discriminated against by health workers. Conclusion: A comprehensive package of interventions, tailored to the local context, will be needed to address the multiple causes of discrimination identified: basic population-wide health education is unlikely to be effective.

761 PADOVEZE, M. C.; FORTALEZA, C. M. C. B.; FREIRE, M. P.; ASSIS, D. B. DE; MADALOSSO, G.; PELLINI, A. C. G.; CESAR, M. L. V.; PISANI NETO, V.; BELTRAMELLI, M. M.; CHIMARA, E.; FERRAZOLI, L.; TELLES, M. A. DA S.; SAMPAIO, J. L. M.; LEAO, S. C. **Outbreak of surgical infection caused by non-tuberculous mycobacteria in breast implants in Brazil.** *Journal of Hospital Infection* (2007) **67** (2) 161-167 Amsterdam, Netherlands; Elsevier [En, 18 ref.] Centro de Vigilância Epidemiológica 'Prof Alexandre Vranjac', State Health Department, Hospital Infection Division, São Paulo, Brazil. Email: padoveze@hc.unicamp.br

We investigated an outbreak caused by non-tuberculous mycobacteria (NTM) related to breast implant surgery in the city of Campinas, Brazil, by means of a retrospective cohort and molecular epidemiological study. A total of 492 records of individuals having breast surgery in 12 hospitals were evaluated. Twelve isolates were analysed using four different molecular typing methods. There were 14 confirmed cases, 14 possible cases and one probable case. One probable, nine possible and 12 confirmed cases were included in a cohort study; all occurred in eight of the hospitals and the confirmed cases in five. Univariate analysis showed that patients who had had breast reconstruction surgery in hospitals A and B were more likely to have NTM infections. No risk factor was independently associated with NTM infection in the multivariate model. The isolates obtained from patients at each hospital showed different molecular patterns, excluding isolates from hospital C that repeatedly showed the same genotype for approximately one year. In conclusion, this outbreak was caused by polyclonal strains at different institutions, and in one hospital a unique genotype caused most cases. No specific risk factors were found.

763 SARMAN SINGH; SANKAR, M. M.; KRISHNAMOORTHY GOPINATH **High rate of extensively drug-resistant tuberculosis in Indian AIDS patients.** *AIDS* (2007) **21** (17) 2345-2347 Hagerstown, USA; Lippincott Williams & Wilkins [En, 12 ref.] Division of Clinical Microbiology, Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi - 110 029, India.

Fifty-four full-blown AIDS patients suspected of having HIV-tuberculosis co-infection were investigated for the prevalence of extensively drug-resistant (XDR) *Mycobacterium tuberculosis*. Out of the 54 patients, *M. tuberculosis* was isolated from 24 (44.4%). Twelve (50%) isolates of these had resistance to first-line drugs, whereas four (33.33%) were also resistant to second-line drugs. All four patients, in whom XDR *M. tuberculosis* was isolated, died within 2.6 months of diagnosis.

764 ADETlFA, I. M. O.; LUGOS, M. D.; HAMMOND, A.; JEFFRIES, D.; DONKOR, S.; ADEGBOLA, R. A.; HILL, P. C. **Comparison of two interferon gamma release assays in the diagnosis of *Mycobacterium tuberculosis* infection and disease in The Gambia.** *BMC Infectious Diseases* (2007) **7** (122) (25 October 2007) London, UK; BioMed Central Ltd [En, 40 ref.] Bacterial Diseases Programme, Medical Research Council Laboratories, Fajara, Gambia. Email: iadetifa@mrc.gm.mlugos2003@yahoo.com. ahammond@mrc.gm, djeffries@mrc.gm, sdonkor@mrc.gm, radegbola@mrc.gm, phill@mrc.gm

Background - IFN- Release Assays (IGRAs) have been licensed for the diagnosis of latent *Mycobacterium tuberculosis* infection (LTBI). Their performance may depend on assay format and may vary across populations and settings. We compared the diagnostic performance of an in-house T -cell and commercial whole blood-based IGRAs for the diagnosis of LTBI and TB disease in The Gambia. Methods - Newly diagnosed sputum smear positive cases and their household contacts were recruited. Cases and contacts were bled for IGRA and contacts had a Mantoux skin test. We assessed agreement and discordance between the tests and categorized a contact's level of *M. tuberculosis* exposure according to where s/he slept relative to a case: the same room, same house or a different house. We assessed the relationship between exposure and test results by multiple logistic regression. Results - In 80 newly diagnosed TB cases, the sensitivity of ELISPOT was 78.7% and for QFT-GITwas 64.0% (p=0.047). Of 194 household contacts 57.1 % and 58.8% were positive for ELlSPOT and QFT-GIT respectively. The overall agreement between both IGRAs for LTBI in contacts was 71.4% and there was no significant discordance (p=0.29). There was significant discordance between the IGRAs and TST. Neither IGRA nor TST had evidence of false positive results because of Bacille Calmette Guerin (BCG) vaccination. However, agreement between QFT-GIT and TST as well as discordance between both IGRAs and TST were associated with BCG vaccination. Both IGRAs responded to the *M.tuberculosis* exposure gradient and were positively associated with increasing TST induration (p=0.003 for ELlSPOT and p=0.001 for QFT-GIT). Conclusions - The ELlSPOT test is more sensitive than the QFT-GIT for diagnosing TB disease. The two tests perform similarly in the diagnosis of LTBI in TB contacts. Significant discordance between the two IGRAs and between each and the TST remains largely unexplained.

766 GHOSHAL, U. C.; GHOSHAL, U.; SINGH, H.; TIWARI, S. **Anti-*Saccharomyces cerevisiae* antibody is not useful to differentiate between Crohn's disease and intestinal tuberculosis in India.** *Journal of Postgraduate Medicine* (2007) **53** (3) 166-170 Mumbai, India; Medknow Publications (En, 35 ref.] Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow - 226 014, India. Email: ghoshal@sgpgi.ac.in

Context: Clinical, endoscopic, radiological and histological parameters of intestinal tuberculosis (IT) and Crohn's disease (CD) are so similar that differentiation between these two diseases, which require different treatment, is difficult. Anti- Saccharomyces cerevisiae antibody (ASCA), which is often present in the sera of patients with CD, may be potentially useful to differentiate CD from IT. Aim: To evaluate the role of enzyme-linked immunosorbent assay test for ASCA in serum in differentiating CD from intestinal tuberculosis. Settings and Design: Prospective case-control study. Materials and Methods: Sixteen patients with IT, 16 CD, 36 UC diagnosed using standard parameters and 12 controls (11 healthy subjects and one with colonic carcinoma) were tested for IgG ASCA in serum. Statistical Analysis Used: Categorical variables were analyzed using Chi-square test with Yates' correction, as applicable. Continuous variables were analyzed using Mann-Whitney U test. Results: Eight of 16 (50%) patients with IT, 10 of 16 with CD (62%), nine of 35 with UC (26%) and one of 12 controls tested positive for ASCA in serum. Though the frequency of ASCA in serum was comparable among patients with IT and CD (8/16 vs. 10/16, *P*=ns), IT and UC (8/16 vs. 9/35, *P*=ns), CD and UC (10/16 vs. 9/35, *P*=ns), its frequency in CD or IT but not in UC was higher than healthy controls (*P*<0.01). Conclusions: Serum ASCA is unlikely to be useful to differentiate between CD and IT in India.

767 SHEARS, **P. Poverty and infection in the developing world: healthcare-related infections and infection control in the tropics.** *Journal of Hospital Infection* (2007) **67** (3) 217-224 Amsterdam, Netherlands; Elsevier (En, 44 ref.] Department of Medical Microbiology, Sheffield Teaching Hospitals NHS Foundation Trust, Glossop Road, Sheffield S10 2JF, UK. Email: Paul.Shears@sth,nhs.uk

In many hospitals serving the poorest communities of Africa and other parts of the developing world, infection control activities are limited by poor infrastructure, overcrowding, inadequate hygiene and water supply, poorly functioning laboratory services and a shortage of trained staff. Hospital transmission of communicable diseases, a high prevalence of human immunodeficiency virus and multidrug-resistant tuberculosis, lack of resources for isolation and disinfection, and widespread antimicrobial resistance create major risks for healthcare-related infections. Few data exist on the prevalence or impact of these infections in such environments. There is a need for interventions to reduce the burden of healthcare-related infections in the tropics and to set up effective surveillance programmes to determine their impact. Both the Global (G8) International Development Summit of 2005 and the United Nations Millennium Development Goals (MDGs) have committed major resources to alleviating poverty and poor health in the developing world over the next decade. Targeting resources specifically to infection control in low-resource settings must be a part of this effort, if the wider aims of the MDGs to improve healthcare e to be achieved.

768 CAMBANIS, A.; RAMSAY, A.; YASSIN, M. A.; CUEVAS, L. E. **Duration and associated factors of patient delay during tuberculosis screening in rural Cameroon.** *Tropical Medicine and International Health* (2007) **12** (11) 1309-1314 Oxford, UK; Blackwell Publishing (En, 32 ref.] St. Elizabeth General Hospital, Shisong, Cameroon. Email: alexiscambanis@yahoo.com

Objectives: (i) To determine patient delay - the time from the onset of symptoms to presentation at a health facility - and its causes in patients undergoing sputum smear examination in Cameroon; and (ii) to compare the results with those of a previous study in Ethiopia. Methods: A cross-sectional study of 243 consecutive patients using a structured questionnaire. Results: Median (interquartile range) patient delay in Cameroon was 2.0 (1-4) weeks, shorter than the 4.3 (2-13) week delay in Ethiopia. Significantly fewer patients delayed more than 1, 2 and 3 months in Cameroon than in Ethiopia (P<0.001). Delays in Cameroon were significantly associated with being the main income earner, the belief that TB is stigmatizing, and the use of traditional medicinethe latter being the only factor significant in both studies. Conclusion: Engaging traditional healers in TB control programs and reducing stigma through education could help to reduce patient delays, accelerate diagnosis, improve clinical outcomes and reduce disease transmission. These results, when placed in context of national human development indices, suggest that economic development, investment in health care and literacy may all be involved in improving access to TB services in sub-Saharan Africa.

769 SOUSA, A. L. O. M.; STEFANI, M. M. A.; PEREIRA, G. A. S.; COSTA, M. B.; REBELLO, P. F.; GOMES, M. K.; NARAHASHI, K.; GILLIS, T. P.; KRAHENBUHL, J. L.; MARTELLI, C. M. T. ***Mycobacterium leprae* DNA associated with type 1 reactions in single lesion paucibacillary leprosy treated with single dose rifampin, ofloxacin, and minocycline.** *American Journal of Tropical Medicine and Hygiene* (2007) **77** (5) 829-833 Northbrook, USA; American Society of Tropical Medicine and Hygiene [En, 22 ref.] Tropical Pathology and Public Health Institute, Federal University of Goias, Rua 235 esq. c/l[a] Avenida, S/N. Setor Universitario, Goiania, Goias, CEP 74605-050, Brazil. Email: almaroclo@dm.com.br, mstefani@iptsp.ufg.br, gis-ner@terra.com.br, mbarcelos@cultura.com.br, epi@fuam.am.gov.br, mkgomes@gmail.com, knarahashi@uol.com.br, tgillis@lsu.edu, celina@iptsp.ufg.br

Leprosy affects skin and peripheral nerves, and acute inflammatory type 1 reactions (reversal reaction) can cause neurologic impairment and disabilities. Single skin lesion paucibacillary leprosy volunteers (N=135) recruited in three Brazilian endemic regions, treated with single-dose rifampin, ofloxacin, and minocycline (ROM), were monitored for 3 years. Poor outcome was defined as type I reactions with or without neuritis. IgM anti-phenolic glycolipid I, histopathology, Mitsuda test, and *Mycobacterium leprae* DNA polymerase chain reaction (ML-PCR) were performed at baseline. ^{2} test, Kaplan-Meir curves, and Cox proportional hazards were applied. The majority of volunteers were adults with a mean age of 30.5±15.4 years; 44.4% were ML-PCR positive. During follow-up, 14.8% of the patients had a poor clinical outcome, classified as a type I reaction. Older age (40 years), ML-PCR positivity, and lesion size >5 cm were associated with increased risk. In multivariate analysis, age (40 years) and ML-PCR positivity remained baseline predictors of type 1 reaction among monolesion leprosy patients.

770 NACKERS, F.; JOHNSON, R. C.; GLYNN, J. R.; ZINSOU, C.; TONGLET, R.; PORTAELS, F. **Environmental and health-related risk factors for *Mycobacterium ulcerans* disease (Buruli ulcer) in Benin.** *American Journal of Tropical Medicine and Hygiene* (2007) **77** (5) 834-836 Northbrook,

USA; American Society of Tropical Medicine and Hygiene [En, 8 ref.] Epidemiology Unit, Université catholique de Louvain, Ecole de Santé Publique, Clos Chapelle-aux-champs 30/EPID3058, 1200 Woluwé-Saint-Lambert, Brussels, Belgium. Email: Fabienne.Nackers@epid.ucl.ac.be, rochjohnson@yahoo.fr, Judith.Glynn @lshtm.ac.uk, portaels@itg.be

We conducted a case-control study between August 2002 and August 2003 to investigate the association between Buruli ulcer (BU) and environmental- and health-related behaviours in southern Benin. Hospital BU cases (*N*=324) and sex- and age-matched neighbourhood controls (*N*=1,173) answered a questionnaire. Regular use of soap for washing, treating injuries with soap or antibiotic powder, and frequent contact with flowing water appeared protective against BU.

771 MOH, R.; DANEL, C.; MESSOU, E.; OUASSA, T.; GABILLARD, D.; ANZIAN, A.; ABO YAO; SALAMON, R.; BISSAGNENE, E.; SEYLER, C.; EHOLIÈ. S.; ANGLARET. X. **Incidence and determinants of mortality and morbidity following early antiretroviral therapy initiation in HIV-infected adults in West Africa.** *AIDS* (2007) **21** (18) 2483-2491 Hagerstown, USA; Lippincott Williams & Wilkins [En, 34 ref.] Trivacan ANRS 1269 Study Group, Abidjan, Côte d'Ivoire. Email: Xavier.Anglaret@isped.u-bordeaux2.fr

Objective: To estimate the incidence and risk factors of mortality and severe morbidity during the first months following antiretroviral therapy (ART) initation in West African adults. Methods: A cohort study in Abidjan in which 792 adults started ART with a median CD4 cell count of 252 cells/µl and were followed for a median of 8 months. Severe morbidity was defined as all World Health Organization stage 3 or 4-defining morbidity events other than oral candidiasis. Results: In patients with pre-ART CD4 cell count <200, at 200-350 and >350 cells/µl, incidence of mortality was 5.0 [95% confidence interval (CI), 2.6-8.7], 1.7 (95% CI, 0.6-3.8) and 0.0 (95% CI, 0.0-3.4/100) person-years, and incidence of severe morbidity was 13.3 (95% CI, 9.0-19.1), 9.5 (95% CI, 6.2-12.9) and 7.9 (95% CI, 3.4-15.5)/100 person-years, respectively. The most frequent diseases were invasive bacterial diseases (32/65 episodes, 49%) and tuberculosis (25/65 episodes, 38%). Both diseases followed the same curve of decreasing incidence over time. Patients who experienced severe morbidity had higher risks of mortality, virological failure and immunological failure. Other independent risk factors for mortality and/or severe morbidity were: at baseline, high viral load, advanced clinical stage, past history of tuberculosis, low BMI, low haemoglobin and low CD4 cell count; during follow-up: low CD4 cell count and persistently detectable viral load. Conclusion: These data give new arguments to reinforce the hypothesis that, in this region, ART should be started before the CD4 cell count drops below 350 cells/µl. Further studies should assess whether patients with low BMI, low haemoglobin, high viral load or past history of tuberculosis should start ART earlier.

1734 SCHERER, L. C.; SPERHACKE, R. D.; JARCZEWSKI, C.; CAFRUNE, P. I.; MINGHELLI, S.; RIBEIRO, M. O.; MELLO, F. C. Q.; RUFFINO-NETIO, A; ROSSETII, M. L. R.; KRITSKI, A. L. **PCR colorimetric dot-blot assay and clinical pretest probability for diagnosis of pulmonary tuberculosis in smear-negative patients.** *BMC Public Health* (2007) **7** (356) (20 December 2007) London, UK; BioMed Central Ltd (En, 34 ref.] Programa de pós Graduacão em Ciências Biológicas- Bioquímica, Universidade

Federal do Rio Grande do Sul-UFRGS, Porto Alegre, RS, Brazil. Email: luciene.scherer@hotmail.com, deasperhacke@hotmail.com, jarczews@terra.com.br, patricia_cafrune@hotmail.com, sminghelli@terra.com.br, martaoso@terra.com.br, fcqmello@hucff.ufrj.br, aruffino@fmrp.usp.br, mrossett@terra.com.br, kritskia@gmail.com

Background: Smear-negative pulmonary tuberculosis (SNPTB) accounts for 30% of Pulmonary Tuberculosis (PTB) cases reported annually in developing nations. Polymerase chain reaction (PCR) may provide an alternative for the rapid detection of *Mycobacterium tuberculosis* (MTB); however little data are available regarding the clinical utility of PCR in SNPTB, in a setting with a high burden of TB/HIV co-infection. Methods: To evaluate the performance of the PCR dot-blot in parallel with pretest probability (Clinical Suspicion) in patients suspected of having SNPTB, a prospective study of 213 individuals with clinical and radiological suspicion of SNPTB was carried out from May 2003 to May 2004, in a TB/HIV reference hospital. Respiratory specialists estimated the pretest probability of active disease into high, intermediate, low categories. Expectorated sputum was examined by direct microscopy (Ziehl-Neelsen staining), culture (Lowenstein Jensen) and PCR dot-blot. Gold standard was based on culture positivity combined with the clinical definition of PTB. Results: In smear-negative and HIV subjects, active PTB was diagnosed in 28.4% (43/151) and 42.2% (19/45), respectively. In the high, intermediate and low pretest probability categories active PTB was diagnosed in 67.4% (31/46), 24% (6/25), 7.5% (6/80), respectively. PCR had sensitivity of 65% (CI 95%: 50%-78%) and specificity of 83% (CI 95%: 75%-89%). There was no difference in the sensitivity of PCR in relation to HIV status. PCR sensitivity and specificity among non-previously TB treated and those treated in the past were, respectively: 69%, 43%, 85% and 80%. The high pretest probability, when used as a diagnostic test, had sensitivity of 72% (CI 95%:57%-84%) and specificity of 86% (CI 95%:78%-92%). Using the PCR dot-blot in parallel with high pretest probability as a diagnostic test, sensitivity, specificity, positive and negative predictive values were: 90%, 71%, 75%, and 88%, respectively. Among non-previously TB treated and HIV subjects, this approach had sensitivity, specificity, positive and negative predictive values of 91 %,79%,81 %,90%, and 90%, 65%, 72%: 88%, respectively. Conclusion: PCR dot-blot associated with a high clinical suspicion may provide an important contribution to the diagnosis of SNPTB mainly in patients that have not been previously treated attended at a TB/HIV reference hospital.

1735 KUCUKARDALI, Y.; ONCUL, O.; CAVUSLU, S.; DANACI, M.; CALANGU, S.; ERDEM, H.; TOPCU, A. W.; ADIBELLI, Z.; AKOVA, M.; KARAALI, E. A.; OZEL, A. M.; BOLAMAN, Z.; CAKA, B.; CETIN. B.; COBAN, E.; KARABAY, O.; KARAKOC, C.; KARAN, M. A.; KORKMAZ. S.; SAHIN, G. O.; PAHSA, A.; SIRMATEL, F.; SOLMAZGUL, E.; OZMEN, N.; TOKATLI, I.; UZUN, C. (ET AL); TURKEY, FEVER OF UNKNOWN ORIGIN STUDY GROUP **The spectrum of diseases causing fever of unknown origin in Turkey: a multicenter study.** *International Journal of Infectious Diseases* (2008) **12** (1) 71-79 Oxford, UK; Elsevier (En, 31 ref.] Department of Internal Medicine, GATA Haydarpasa Hospital, Istanbul, Turkey. Email: hakanerdem1969@yahoo.com

Objective: The purpose of this trial was to determine the spectrum of diseases with fever of unknown origin (FDO) in Turkey.

Methods: A prospective multicenter study of 154 patients with FUO in twelve Turkish tertiary-care hospitals was conducted. Results: The mean age of the patients was 42±17 years (range 17-75). Fifty-three (34.4%) had infectious diseases (ID), 47 (30.5%) had non-infectious inflammatory diseases (NIID), 22 (14.3%) had malignant diseases (MD), and eight (5.2%) had miscellaneous dis-eases (Mi). In 24 (15.6%) of the cases, the reason for high fever could not be determined despite intensive efforts. The most common 10 etiologies were tuberculosis (13.6%) and cytomegalovirus (CMV) infection (3.2%). Adult Still's disease was the most common NIID (13.6%) and hematological malignancy was the most common MD (7.8%). In patients with NIID, the mean duration of reaching a definite diagnosis (37±23 days) was significantly longer compared to the patients with 10 (25±12 days) (p=0.007). In patients with MD, the mean duration of fever (51±35 days) was longer compared to patients with 10 (37±38 days) (p=0.052). Conclusions: Although infection remains the most common cause of FUO, with the highest percentage for tuberculosis, non-infectious etiologies seem to have increased when compared with previous studies.

1736 SCHAAF, H. S.; VICTOR, T. C.; ENGELKE, E.; BRITTLE, W.; MARAIS, B. J.: HESSELING, A. C.; HELDEN, P. D. VAN; DONALD, P. R. **Minimal inhibitory concentration of isoniazid in isoniazid-resistant *Mycobacterium tuberculosis* isolates from children.** *European Journal of Clinical Microbiology & Infectious Diseases* (2007) **26** (3) 203-205 Berlin, Germany; Springer-Verlag GmbH [En] Desmond Tutu Tuberculosis Centre, Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, P.O. Box 19063, Tygerberg, 7505, South Africa. Email: hss@sun.ac.za

The aim of the study presented here was to determine the minimal inhibitory concentration of isoniazid for strains of isoniazidresistant or multidrug-resistant *Mycobacterium tuberculosis* isolated from children in the Western Cape Province of South Africa. During the period March 2003-0ctober 2005, 45 INH-resistant *M. tuberculosis* isolates (21 also rifampicin-resistant) were cultured from children less than 13 years of age. Drug susceptibility testing by the radiometric BACTEC 460 method found 11 isolates resistant at 0.1 μg/ml, 27 resistant at 0.2 μg/ml, and seven resistant at 5 μg/ml. Thus, the minimal inhibitory concentration of isoniazid for more than 80% of the isoniazid-resistant strains isolated from children in this study was relatively low and could be exceeded by high-dose (15-20 mg/kg) isoniazid regimens.

1737 COHEN, K.; CUTSEM, G. VAN; BOULLE, A.; McILLERON, H.; GOEMAERE, E.; SMITH, P. J.; MAARTENS, G. **Effect of rifampicin-based antitubercular therapy on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis.** *Journal of Antimicrobial Chemotherapy* (2008) **61** (2) 389-393 Oxford, UK; Oxford University Press [En] Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa. Email: karen.cohen@uct.ac.za

Background and objectives: Nevirapine-containing antiretroviral therapy (ART) and rifampicin-based antitubercular therapy are commonly co-administered in Africa, where nevirapine is often the only available non-nucleoside reverse transcriptase inhibitor. Rifampicin induces the metabolism of nevirapine, but the extent of the reduction in nevirapine concentrations has varied widely in previous studies. We describe the steady-

state pharmacokinetics of nevirapine during and after antitubercular therapy in South African patients. Methods: Sixteen patients receiving ART including standard doses of nevirapine were admitted twice for intensive pharmacokinetic sampling: during and after rifampicin-based antitubercular therapy. Results: Geometric mean ratios for nevi rapine pharmacokinetic parameters during versus after antitubercular therapy were 0.61 [90% confidence interval (CI) 0.49-0.79] for C_{max}, 0.64 (90% CI 0.52-0.80) for area under the curve up to 12 h (AUC_{0-12}) and 0.68 (90% CI 0.53-0.86) for C_{min}. Nevirapine C_{min} was subtherapeutic (<3 mg/L) in six patients during antitubercular therapy (one of whom developed virological failure) and in none afterwards. There was no correlation between rifampicin concentrations and the degree of nevirapine induction assessed by the proportional change in nevirapine concentrations between the two admissions. The ratio of nevirapine AUC_{0-12} to the AUC_{0-12} of its 12-hydroxy metabolite was significantly lower in the presence of antitubercular therapy, consistent with induced metabolism. Conclusions: Nevirapine concentrations were significantly decreased by concomitant rifampicin-based antitubercular therapy and a high proportion of patients had subtherapeutic plasma concentrations. Further study in African patients is required to determine the implications for treatment outcomes.

1738 HERNANDEZ, J.; GARIBAY-ESCOBAR, A; MENDOZA-MENDOZA, A.; PINELLl-SAAVEDRA, A.; VALENZUELA, O. **Effect of exogenous vitamin E on proliferation and cytokine production in peripheral blood mononuclear cells from patients with tuberculosis.** *British Journal of Nutrition* (2008) **99** (2) 224-229 Cambridge, UK; Cambridge University Press [En] Laboratorio de Inmunología, Centro de Investigación en Alimentación y Desarrollo, A. C. Carretera a la Victoria Km 0.6. Hermosillo, 83000, Sonora, Mexico. Email: jhdez@ciad.mx

Micronutrient deficiencies are frequently associated with tuberculosis (TB) worldwide. We tested the effect of exogenous vitamin E on proliferation and cytokine production of peripheral blood mononuclear cells (PBMC) from TB patients and healthy purified protein derivative (PPD)+ volunteers. Proliferation was stimulated with mycobacterial antigen (PPD) and evaluated by the incorporation of tritiated thymidine in PBMC cultured with or without 50 µm-vitamin E for 6 d. Cytokine production (IL-2 and interferon (IFN-) was determined by intracellular cytokine staining and by ELISA in the supernatant of PBMC stimulated for 24 h with phytohaemagglutinin or PPD. Our results show that culture with vitamin E increased (*P* 0.05) the antigen-induced proliferation of PBMC in TB patients but not in healthy PPD+ volunteers. No significant changes in the number of cytokine-producing cells or in the production of IFN- were observed with vitamin E treatment. These results indicate that vitamin E may enhance the antigen-specific *in vitro* response of PBMC from TB patients.

1739 SAGBAKKEN, M.; FRICH, J. C.; BJUNE, G. **Barriers and enablers in the management of tuberculosis treatment in Addis Ababa, Ethiopia: a qualitative study.** *BMC Public Health* (2008) **8** (11) (11 January 2008) London, UK; BioMed Central Ltd [En, 46 ref.] Section for International Health, Institute of General Practice and Community Medicine, University of Oslo, P.O. Box 1130 Blindern, NO-0318 Oslo, Norway. Email: mette.sagbakken@medisin.uio.no, j.c.d.frich@medisin.uio.no, g.a.bjune@medisin.uio.no

Background: Non-adherence to

tuberculosis (TB) treatment is an important barrier for TB control programs because incomplete treatment may result in prolonged infectiousness, drug resistance, relapse, and death. The aim of the present study is to explore enablers and barriers in the management of TB treatment during the first five months of treatment in Addis Ababa, Ethiopia. Methods: Qualitative study which included 50 in-depth interviews and two focus groups with TB patients, their relatives and health personnel. Results: We found that loss of employment or the possibility to work led to a chain of interrelated barriers for most TB patients. Daily treatment was time-consuming and physically demanding, and rigid routines at health clinics reinforced many of the emerging problems. Patients with limited access to financial or practical help from relatives or friends experienced that the total costs of attending treatment exceeded their available resources. This was a barrier to adherence already during early stages of treatment. A large group of patients still managed to continue treatment, mainly because relatives or community members provided food, encouragement and sometimes money for transport. Lack of income over time, combined with daily accumulating costs and other struggles, made patients vulnerable to interruption during later stages of treatment. Patients who were poor due to illness or slow progression, and who did not manage to restore their health and social status, were particularly vulnerable to non-adherence. Such patients lost access to essential financial and practical support over time, often because relatives and friends were financially and socially exhausted by supporting them. Conclusion: Patients' ability to manage TB treatment is a product of dynamic processes, in which social and economic costs and other burdens change and interplay over time. Intervention to facilitate adherence to TB treatment needs to address both time-specific and local factors.

1740 RIE, A. VAN; SENGUPTA, S.; PUNGRASSAMI, P.; BALTHIP, Q.; CHOONUAN, S.; KASETJAROEN, Y.; STRAUSS, R. P.; CHONGSUVIVATWONG, V. **Measuring stigma associated with tuberculosis and HIV/AIDS in southern Thailand: exploratory and confirmatory factor analyses of two new scales.** *Tropical Medicine and International Health* (2008) **13** (1) 21-30 Oxford, UK; Blackwell Publishing [En, fr, es, many ref.] The University of North Carolina, Chapel Hill, NC 27514, USA. Email: vanrie@email.unc.edu

Objective: To develop scales to measure tuberculosis and HIV/AIDS stigma in a developing world context. Methods: Cross-sectional study of tuberculosis patients in southern Thailand, who were asked to rate their agreement with items measuring TB and HIV/AIDS stigma. Developing the scales involved exploratory and confirmatory factor analyses, internal consistency, construct validity, test-retest reliability and standardized summary scores. Results: Factor analyses identified two sub-scales associated with both tuberculosis and HIV/AIDS stigma: community and patient perspectives. Goodness-of-fit was good (TLI=94, LFI=0.88 and RMSEA=0.11), internal consistency was excellent (Cronbach's alphas 0.82-0.91), test-retest reliability was moderate, and construct validity showed an inverse correlation with social support. Conclusion: Our scales have good psychometric properties that measure stigma associated with tuberculosis and HIV/AIDS and allow assessment of stigma from community and patient perspectives. Their use will help document the burden of stigma, guide the development of interventions and evaluate stigma reduction

grammes in areas with a high HIV/AIDS and tuberculosis burden.

1741 MOREIRA, J.; ALARCON, F; BISOFFI, Z.; RIVERA. J.; SALINAS, R.; MENTEN, J.; DUENAS, G.; ENDE, J. VAN DEN **Tuberculous meningitis: does lowering the treatment threshold result in many more treated patients?** *Tropical Medicine and International Health* (2008) **13** (1) 68-75 Oxford, UK; Blackwell Publishing [En, es, fr, 35 ref.] Institute of Tropical Medicine, Antwerp, Belgium. Email: alyosha@access.net.ec. falarcon@ramt.com

Objective: To determine how many more patients would be treated when lowering the treatment threshold for tuberculous meningitis. Methods: From 1989 to 2004 findings of patients with symptoms lasting more than 1 week and inflammatory changes of cerebrospinal fluid (CSF) were collected. Several models of latent class analysis were tested. Cumulative numbers of cases were plotted against different cut-offs for post-test probability. Results: In a cohort of 232 patients the prevalence of tuberculous meningitis (TBM) was estimated at 79.8% (95% CI. 67,0-88,1); probabilities above 80% were reached in 73% of patients. Lowering this threshold from 80% to 20% would add 14% more patients to be treated, for a total of 87%. A further lowering of the threshold to 5% would imply 5% more patients to be treated, bringing the cumulative number to 92%. The difference of lowering the threshold from 80% to 5% was 19%. Conclusion: In this setting, at least 75% of patients showing suggestive symptoms for more than a week and CSF changes very probably had TBM. The number of patients that should be treated does not increase linearly when lowering the threshold.

1742 GEGIA, M.; MDIVANI, N.; MENDES, R. E.; LI, H. J.; AKHALAIA, M.; HAN, J.; KHECHINASHVILI, G.; TANG, Y. W. **Prevalence of and molecular basis for tuberculosis drug resistance in the Republic of Georgia: validation of a QIAplex system for detection of drug resistance-related mutations.** *Antimicrobial Agents and Chemotherapy* (2008) **52** (2) 725-729 Washington, USA; American Society for Microbiology (ASM) [En, 17 ref.] Georgian Foundation against Tuberculosis and Lung Diseases, Tbilisi, Georgia, USA. Email: yiwei.tang@vanderbilt.edu

We developed a QIAplex system for the simultaneous detection of 24 *Mycobacterium tuberculosis* gene mutations responsible for resistance to isoniazid (INH), rifampin (RIF), streptomycin (STM), and ethambutol (EMB) in 196 *M. tuberculosis* isolates recovered in the Republic of Georgia. In comparison to phenotypic susceptibility tests, the QIAplex showed sensitivity and specificity of 85.4% and 96.1 % for INH, 94.4% and 99.4% for RIF, 69.6% and 99.2% for STM, 50.0% and 98.8% for EBM, and 86.7% and 100.0% for multidrug resistance, respectively. The dominant resistance mutations revealed were a mutation in *katG* resulting in S315T (*katG* S315T), *rpsL* K43R, and *rpoB* S531L. Mutations *katG* S315G and S315T and *rpoB* S531L were detected with higher frequencies in pretreated patients than in naive patients ($P<0.05$). Simultaneous detection of 24 common drug resistance-related mutations provides a molecular tool for studying and monitoring *M. tuberculosis* resistance mechanism and epidemiology.

1743 CARSALADE, G. Y. DE; RECEVEUR, M. C.; EZZEDINE, K.; SAGET, J.; ACHIRAFI, A.; BOBIN, P.; MALVY. D. **[Delayed home screening of leprosy; experience of the screening team in Mayotte.]** Dépistage actif intra-domiciliaire différé de la lèpre: expérience de l'équipe de dépistage sur l'île

de Mayotte. *Bulletin de la Société de Pathologie Exotique* (2008) **101** (I) 32-35 Paris, France; Société de Pathologie Exotique [Fr, en, 9 ref.] Service des urgences, Centre hospitalier de Mayotte, 97600 Mamoudzou, Mayotte. Email: kezzedin@ulb.ac.be

A delayed active screening of household contacts of leprosy cases in Mayotte, Comoros, who were registered by passive detection between 1997 and 2003, was performed in 2003. A total of 325 household contacts were examined and 15 new cases were detected. All these new cases showed early leprosy features: 14 had paucibacillary leprosy, 9 of which had an isolated cutaneous lesion (7 had an infracentimetric lesion). One patient had multi bacillary leprosy although he presented with an isolated skin lesion that developed within the 6 previous months. None presented with disability. These results suggest that passive detection even reinforced by repeated individual information and education about leprosy is neither appropriate nor effective. Delayed active screening seems to favour an increased self-esteem and a better involvement of the index patient in sanitation education and screening of relatives. In Mayotte, delayed active screening has not been associated with a significant delay in diagnosis. Although WHO recommended to abandon immediate active screening of household contacts and to promote self-screening for leprosy, this study suggests an intermediate position, that is, delayed active screening for an enhanced effective detection.

1744 EOOYANl, M.; DEBACKER, M.; MARTIN, A.; AGUIAR, J.; JOHNSON, C. R.; UWIZEYE, C.; FISSETTE, K.; PORTAELS, F. **Primary culture of *Mycobacterium ulcerans* from human tissue specimens after storage in semisolid transport medium.** *Journal of Clinical Microbiology* (2008) **46** (1) 69-72 Washington, USA; American Society for Microbiology (ASM) [En, 18 ref.] Mycobacteriology Unit, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium. Email: meddyani@itg.be

Tissue specimens collected from patients with clinically suspected Buruli ulcer treated in two Buruli ulcer treatment centers in Benin between 1998 and 2004 were placed in semisolid transport medium and transported at ambient temperature for microbiological analysis at the Institute of Tropical Medicine in Antwerp, Belgium. The impact of the delay before microbiological analysis on primary culture of *Mycobacterium ulcerans* was investigated. The length of storage in semisolid transport medium varied from 6 days to 26 weeks. Of the 1,273 tissue fragments positive for *M. ulcerans* DNA by an IS*2404*-specific PCR, 576 (45.2%) yielded positive culture results. The sensitivity of direct smear examination was 64.6% (822/1,273 tissue fragments). The median time required to obtain a positive culture result was 11 weeks. Positive cultures were obtained even from samples kept for more than 2 months at ambient temperatures. Moreover, there was no reduction in the viability of *M. ulcerans*, as detected by culture, when specimens remained in semisolid transport medium for long periods of time (up to 26 weeks). We can conclude that the method with semisolid transport medium is very robust for clinical specimens from patients with Buruli ulcer that, due to circumstances, cannot be analyzed in a timely manner. This transport medium is thus very useful for the confirmation of a diagnosis of Buruli ulcer with specimens collected in the field.

1745 NUZHAT HUSAIN; SEEMA AWASTHl; MOHO HARIS; GUPTA, R. K.; MAZHAR HUSAIN **Vascular endothelial growth factor as a marker of disease activity in neurotuberculosis.** *Journal of Infection*

(2008) **56** (2) 114-119 Amsterdam, Netherlands; Elsevier [En, 16 ref.] Department of Pathology, King George's Medical University, Lucknow, 226 003, India. Email: drnuzhathusain@hotmail.com

Vascular endothelial growth factor (VEGF) is a potent angiogenesis mediator. Scant reports are available defining the role of VEGF in active and inactive tubercular meningitis (TBM) with no studies on brain tuberculoma. We quantified VEGF levels by enzyme linked immunoassay (ELISA) in cerebrospinal fluid (CSF) and serum in 20 cases each with active and inactive TBM as well as 22 cases of intraparenchymal tuberculoma. VEGF expression and microvessel angiogenesis quantification was done in 7 cases where tuberculomas were excised. Significantly increased VEGF levels in CSF were found in active TBM cases (106.0±50.0 pg/ml) compared to inactive TBM cases (14.7±10.0 pg/ml) (p<0.001). Mean serum VEGF levels in active TBM, inactive TBM and tuberculoma were 694.93±820.66 pg/ml, 499.61±238.33 pg/ml and 541.0±389.0 pg/ml, respectively. Immunohistochemical staining of excised tuberculoma demonstrated high expression of VEGF in granulomatous areas with intense positivity in inflammatory mononuclear cells, Langhan's giant cells as well as reactive astrocytes and fibrocytes. A strong positive correlation was observed between microvessel density and VEGF expression. Serial decrease in serum VEGF levels was observed with increasing duration of therapy in tuberculoma. We conclude that increased CSF and serum VEGF levels are a measure of activity of the disease in neurotuberculosis and its gradual decrease over a period of time is probably an indicator of therapeutic response.

1746 AFFOLABI, D.; BANKOLE, H.; ABLORDEY, A.; HOUNNOUGA, J.; KOUTCHAKPO, P.; SOPOH, G.; AGUIAR, J.; DOSSOU, A.; JOHNSON, R. C.; ANAGONOU. S.; PORTAELS, F. **Effects of grinding surgical tissue specimens and smear staining methods on Buruli ulcer microscopic diagnosis.** *Tropical Medicine and International Health* (2008) **13** (2) 187-190 Oxford, UK; Blackwell Publishing [En, fr, es, 13 ref.] Laboratoire de Reference des Mycobacteries, Cotonou, Benin. Email: portaels@itg.be

To optimize Buruli ulcer (BU) microscopic diagnosis, we compared two smear preparation methods from tissue specimens: smears made with tissue suspension after grinding and smears made directly with unground tissue. We also compared two smear staining methods: auramine and Ziehl-Neelsen (ZN). IS 2404-PCR was used as reference method. One hundred and thirty-one surgical tissue specimens from patients suspected of having BU were analyzed. Both smear preparation methods and both staining methods were equivalent in any combination. Thus we recommend ZN stained smears of unground tissue for peripheral treatment centres.

1747 MENSAH-QUAINOO, E.; YEBOAH-MANU, D.; ASEBI, C.; PATAFUOR, F.; OFORI-ADJEI, D.; JUNGHANSS, T.; PLUSCHKE, G. **Diagnosis of *Mycobacterium ulcerans* infection (Buruli ulcer) at a treatment centre in Ghana: a retrospective analysis of laboratory results of clinically diagnosed cases.** *Tropical Medicine and International Health* (2008) **13** (2) 191-198 Oxford, UK; Blackwell Publishing [En, es, fr, 32 ref.] Ghana Health Service, Ministry of Health, Tema, Ghana. Email: gerd. pi uschke@unibas.ch

Clinical diagnosis of *Mycobacterium ulcerans* infection is currently accepted as sufficient basis for treating the disease.

Inadequate laboratory resources in the highly endemic areas of Africa often limit possibilities for in-country confirmation of clinical judgement. We analysed records of 99 Buruli ulcer (BU) patients diagnosed clinically and treated surgically at Amasaman Health Centre in Ghana, for whom post-treatment diagnostic laboratory tests were performed. Comparison of clinical diagnoses with test results obtained by an in-country laboratory on samples of excised tissue showed a high specificity of clinical judgement. Among lesions with three laboratory tests (microscopy for acid fast bacilli, culture and IS2404 polymerase chain reaction) done, 94% tested positive at least once and 83% twice. Thus correct clinical diagnosis of BU by well trained health workers is achievable, although the quality of clinical diagnosis should be monitored by intermittent testing in national reference laboratories. However, being trospective, this study did not permit sensitivity and negative redictive value analysis.

1748 MEIMA, A.; VEEN, N. H. J. VAN; RICHARDUS, J. H. **Future prevalence of WHO grade 2 impairment in relation to incidence trends in leprosy: an exploration.** *Tropical Medicine and International Health* (2008) **13** (2) 241-246 Oxford, UK; Blackwell Publishing [En, es, fr, 18 ref.] Department of Public Health, Erasmus MC, University Medical Center Rotterdam, 3000 CA Rotterdam, Netherlands. Email: n.vanveen@erasmusmc.nl

Objectives: To explore the relationship between leprosy incidence trends and the future prevalence of World Health Organization (WHO) grade 2 impairment caused by leprosy. Methods : Three scenarios were defined to estimate incidences and prevalences of leprosy impairment beyond 2000, assuming 6%, 12% and 18% annual declines in case detection rate respectively, and 6% impairment among new patients. Case detection data from 1985 to 2000 were used for projecting leprosy incidences up to 2020. To estimate future prevalences of WHO grade 2 impairment, the survival of existing and new impaired individuals was calculated. Results : In the 6% scenario, 410 000 new patients will be detected in 2010 and 250 000 in 2020. The number of people living with WHO grade 2 impairment in these years will be 1.3 and 1.1 million, respectively. The 12% scenario predicts that 210 000 new patients will be detected in 2010 and 70 000 in 2020. The grade 2 prevalences will be 1.2 and 0.9 million, respectively. In the 18% scenario, the incidence will be 110 000 in 2010 and 20 000 in 2020, and the grade 2 prevalences will be 1.1 and 0.8 million, respectively. Conclusions : Declines in number of people living with grade 2 impairment lag behind trends in leprosy incidence. The prevalence of people with grade 2 decreases much slower than leprosy incidence and case detection in all three scenarios. This implies that a substantial number of people will live with impairment and will need support, training in self-care and other prevention of disability interventions in the next decades.

1749 DANIEL, E.; RAO, P.S.S.S. **Evolution of vision reducing cataract in skin smear positive lepromatous patients: does it have an inflammatory basis?** *British Journal of Ophthalmology* (2007) **91** (8) 1011-1013 London, UK; BMJ Publishing Group [En] Schieffelin Leprosy Research and Training Centre, Vellore, India. Email: edanie14 @jhmi.edu

Aim: To describe the incidence and risk factors of vision reducing cataract in skin smear positive lepromatous patients. Methods: Prospective longitudinal cohort study: 212 newly diagnosed lepromatous patients were followed during the two years of treatment with multidrug therapy and for

a further five years, with biannual ocular examinations. Incidence of vision reducing (≤6/18) cataract was calculated as the number of patients with cataract per person year of cataract-free follow up among those who did not have cataract at baseline. Results: Cataract was present in 27 (11%) of lepromatous patients at diagnosis. Forty nine patients (2.87%/ person year (95% confidence interval (CI), 2.17% to 3.80%)) developed cataract during a total follow up period of 1704 person years; 45 of these were ≥41 years old and were followed for a total of 638 person years with an incident rate of 0.070 (95% CI, 0.0523 to 0.094). Stepwise multiple regression confirmed the association of age (per decade) (hazard ratio (HR)=2.50 (95% CI, 1.82 to 2.78), p<0.001), clofazimine crystals on the cornea (HR=49.92 (5.48 to 454.82), p=0.001), grade 2 deformity in all limbs (HR=3.17 (1.12 to 8.97), p=0.029), and uveal inflammation (HR=3.52 (1.42 to 8.67), p=0.006). No significant association was found with oral steroids. Conclusions: Cataract develops at the rate of 7%/person year in lepromatous patients over 40 years of age. It is associated with increasing age, subclinical intraocular inflammation, and grade 2 deformity.

1750 PARRADO, R.; LOZANO, D.: GARCIA; L.; TOR RICO, M. C.; DELGADO, R.; TORRICO, F.; LASERNA, M.; REITHINGER, R. **Multiprimer PCR system diagnosis of pulmonary tuberculosis in Cochabamba, Bolivia.** *Journal of Clinical Microbiology* (2008) **46** (2) 830-831 Washington, USA; American Society for Microbiology (ASM) [En, 10 ref.] Instituto de Investigaciones Biomedicas, Facultad de Medicina, Universidad Mayor San Simon, Cochabamba, Bolivia. Email: rupava@gmail.com

A multi primer system polymerase chain reaction (MS-PCR) protocol was used to detect *Mycobacterium* spp. in sputum samples of 33 suspected pulmonary tuberculosis patients (aged> 15 years) attending a regional tuberculosis laboratory in Cochabamba, Bolivia, between July and September 2004. The MS-PCR protocol was compared with routine culture and microscopy. Of the 33 samples tested, 11 (33%),7 (21 %) and II (33%) were positive by MSPCR, microscopy and culture, respectively. Assuming that culture is the diagnostic gold standard, both microscopy and MS-PCR were 100% specific. The sensitivities of MS-PCR and microscopy were 100 and 67%, respectively. The MS-PCR protocol clearly identified all clinical samples positive for *M. tuberculosis*, with the characteristic species- and genus-specific bands readily observed in all cases. It is concluded that the MS-PCR protocol can be readily implemented for the diagnosis of tuberculosis in Bolivia.

1751 ELBIR, H., ABDEL-MUHSIN, A. M., BABIKER, A. **A one step DNA PCR-based method for the detection of *Mycobacterium tuberculosis* complex grown on Lowenstein-Jensen media.** *American Journal of Tropical Medicine and Hygiene* (2008) **78** (2) 316-317 Northbrook. USA; American Society of Tropical Medicine and Hygiene [En, 14 ref.] Department of Microbiology, Tropical Medicine Research Institute, National Center for Research, Khartoum, Sudan. Email: haythamalbur@hotmail.com

A simple, rapid, and sensitive direct colony polymerase chain reaction (PCR) method to detect *Mycobacterium tuberculosis* complex grown on Lowenstein-Jensen media is described. *M. tuberculosis* is killed by treating it for 2 h with 70% ethanol. Whole *Mycobacterium* cells inactivated by ethanol are added to a PCR mix that is designed to amplify the IS6110 insertion sequence. All 44 isolates tested, obtained from tuberculosis patients seen in Khartoum, Sudan, were

positive by this method. Our results show that PCR can be performed directly on bacterial colonies without the need for DNA extraction before PCR. Moreover, inactivation of *M. tuberculosis* before DNA amplification reduces the potential exposure of workers to viable *M. tuberculosis*. The exclusion of DNA extraction and inactivation of colonies before PCR provide a safe and low-cost preparatory technique for PCR reaction compared with expensive conventional extraction protocols that are based on chemical and enzymatic lysis, especially for countries with limited resources.

1752 WRIGHT, C. A.; PIENAAR, J. P.; MARAIS, B. J. **Fine needle aspiration biopsy: diagnostic utility in resource-limited settings.** *Annals of Tropical Paediatrics* (2008) **28** (1) 65-70 Leeds, UK; Maney Publishing [En, 23 ref.] Discipline of Anatomical Pathology, Department of Pathology and NHLS Tygerberg, University of Stellenbosch, PO Box 19063, Tygerberg 7505, South Africa. Email: cawr@sun.ac.za

Background: Little information is available on the value of fine needle aspiration biopsy (FNAB) in routine paediatric practice in resource-limited settings. Aim: To provide an overview of all paediatric FNAB samples received at Tygerberg Hospital, Cape Town, South Africa over a 3-year period, including the determinants of sample adequacy and the diagnoses. Methods: Samples were analysed from three locations: Tygerberg Hospital (TBH) where pathologists performed all the procedures, surrounding clinics where aspirates were mostly performed by doctors with no formal training in FNAB technique, and Queen Elizabeth Hospital, Blantyre, Malawi where FNABs were performed by trained nurse aspirators. Results: A total of 830 aspirates were reviewed: 464 (56%) from TBH, 264 (32%) from local clinics and 102 (12%) from Blantyre. The main diagnoses at TBH were mycobacterial infection (31%), normal/reactive tissue (27%) and malignancy (14%); malignancy dominated (74%) in the select group from Blantyre. Sample adequacy rates were similar between pathologists and nurse aspirators [399/464 vs 82/102, odds ratio (OR) 1.4,95% confidence interval (CI) 0.8-2.6]. Results were significantly better in the group who received formal training (TBH and Malawi) than in the clinics where clinicians had no formal training (481/566 vs 171/264, OR 3.1, 95% CI 2.2-4.4). Conclusions: FNAB provides a definitive tissue diagnosis in the majority of patients. Well-trained nurse aspirators perform as well as pathologists, indicating the feasibility of FNAB in resource-limited settings.

1753 RABAHI, M. F.; JUNQUEIRA-KIPNIS, A. P.; REIS, M. C. G. DOS; OELEMANN, W.; CONDE, M. B. **Humoral response to HspX and GlcB to previous and recent infection by *Mycobacterium tuberculosis*.** *BMC Infectious Diseases* (2007) **7** (148) (31 December 2007) London, UK; BioMed Central Ltd [En, 28 ref.] Departamento de Clinica Medica, Faculdade de Medicina, Universidade Federal de Goins, Goiania, Brazil. Email: mfrabahi@terra.com.br. anapaula @iptsp.ufg.br, michelleguerreiro@ hotmail.com, oelemann@micro.ufrj.br, upt@hucff.ufrj.br

Background: Tuberculosis (TB) remains a major world health problem. Around 2 billions of people are infected by *Mycobacterium tuberculosis*, the causal agent of this disease. This fact accounts for a third of the total world population and it is expected that 9 million people will become infected each year. Only approximately 10% of the infected people will develop disease. However, health care workers (HCW) are continually exposed

to the bacilli at endemic sites presenting increased chance of becoming sick. The objective of this work was to identify LTBI (latent tuberculosis infection) among all asymptomatic HCW of a Brazilian Central Hospital, in a three year follow up, and evaluate the humoral response among HCW with previous and recent LTBI to recombinant HspX and GIcB from *M. tuberculosis.* Methods: Four hundred and thirty seven HCW were screened and classified into three different groups according to tuberculin skin test (TST) status: uninfected, previous LTBI and recent LTBI. ELISA test were performed to determine the humoral immune response to HspX and GlcB. Results: The levels of IgG and IgM against the HspX and GIcB antigens were the same among HCW with recent and previous LTBI, as well as among non infected HCW. However, the IgM levels to HspX was significantly higher among HCW with recent LTBI (OD=1.52±0.40) than among the uninfected (OD=1.09±0.50) or subjects with previous LTBI (OD=0.96±0.51) ($p<0.001$). Conclusion: IgG and IgM humoral responses to GlcB antigens were similar amongst all studied groups; nevertheless IgM levels against HspX were higher among the recent LTBI/HCW.

1754 SREERAMAREDDY, C. T.; PANDURU, K. V.; VERMA, S. C.; JOSHI, H. S.; BATES, M. N. **Comparison of pulmonary and extrapulmonary tuberculosis in Nepal- a hospital-based retrospective study.** *BMC Infectious Diseases* (2008) **8** (8) (24 January 2008) London, UK; BioMed Central Ltd [En, 26 ref.] Department of Community Medicine, Manipal Teaching Hospital, Manipal College of Medical Sciences, Pokhara, Nepal. Email: chandrashek-harats@yahoo.com, pandu_vki @yahoo.com, verma_sharat@yahoo.com, drjoshiharish@rediffmail.com, m_bates@ berkeley.edu

Background: Studies from developed countries have reported on host-related risk factors for extra-pulmonary tuberculosis (EPTB). However, similar studies from high-burden countries like Nepal are lacking. Therefore, we carried out this study to compare demographic. life-style and clinical characteristics between EPTB and PTB patients. Methods: A retrospective analysis was carried out on 474 Tuberculosis (TB) patients diagnosed in a tertiary care hospital in western Nepal. Characteristics of demography, lifestyle and clinical features were obtained from medical case records. Risk factors for being an EPTB patient relative to a PTB patient were identified using logistic regression analysis. Results: The age distribution of the TB patients had a bimodal distribution. The male to female ratio for PTB was 2.29. EPTB was more common at younger ages (<25 years) and in females. Common sites for EPTB were lymph nodes (42.6%) and peritoneum and/or intestines (14.8%). By logistic regression analysis, age less than 25 years (OR 2.11 95% CI 1.12-3.68) and female gender (OR 1.69,95% CI 1.122.56) were associated with EPTB. Smoking, use of immunosuppressive drugs/steroids, diabetes and past history of TB were more likely to be associated with PTB. Conclusion: Results suggest that younger age and female gender may be independent risk factors for EPTB in a high-burden country like Nepal. TB control programmes may target young and female populations for EPTB case-finding. Further studies are necessary in other high-burden countries to conJlrm our findings.

1755 GOLETTI, D.; CARRARA, S.; MAYANJA-KIZZA, H.; BASEKE, J.; MUGERWA, M. A.; GIRARDI, E.; TOOSSI, Z. **Response to *M. tuberculosis* selected RDI peptides in Ugandan HI V-infected patients with smear positive pulmonary tuberculosis: a pilot study.** *BMC Infectious*

Diseases (2008) **8** (11) (28 January 2008) London, UK; BioMed Central Ltd [En, 41 ref.] Translational Research Unit, Department of Experimental Research, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani - IRCCS, Rome, Italy. Email: d.goletti@tiscali.it.carrara@inmi.it. hmk@mucwru.or.ug, immuno@jcrc.co.ug, mmugerwa@mucwru.or.ug, girardi@inmi.it, zxt2@po.cwru.edu

Background: Tuberculosis (TB) is the most frequent co-infection in HIV-infected individuals still presenting diagnostic difficulties particularly in developing countries. Recently an assay based on IFN-gamma response to *M. tuberculosis* RDI peptides selected by computational analysis was developed whose presence is detected during active TB disease. Objective of this study was to investigate the response to selected RDI peptides in HIV-Iinfected subjects with or without active TB in a country endemic for TB and to evaluate the change of this response over time. Methods: 30 HIV-infected individuals were prospectively enrolled, 20 with active TB and 10 without. Among those with TB, 12 were fol-lowed over time. IFN-gamma response to selected RD1 peptides was evaluated by enzyme-linked immunospot (ELlS POT) assay. As control, response to RDI proteins was included. Results were correlated with immune, microbiological and virological data. Results: Among patients with active TB, 2/20 were excluded from the analysis, one due to cell artifacts and the other to unresponsiveness to *M. tuberculosis* antigens. Among those analyzable, response to selected RD I peptides evaluated as spot-forming cells was significantly higher in subjects with active TB compared to those without (p=0.02). Among the 12 TB patients studied over time a significant decrease (p=<0.007) of IFN-gamma response was found at completion of therapy when all the sputum cultures for *M. tuberculosis* were negative. A ratio of RDI peptides ELlSPOT counts over $CD4^+$ T-cell count's greater than 0.21 yielded 100% sensitivity and 80% specificity for active TB. Conversely, response to RD1 intact proteins was not statistically different between subjects with or without TB at the time of recruitment; however a ratio of RD1 proteins ELlS POT counts over $CD4^+$ T-cell counts greater than 0.22 yielded 89% sensitivity and 70% specificity for active TB. Conclusion: In this pilot study the response to selected RD1 peptides is associated with TB disease in HIV infected individuals in a high TB endemic country. This response decreases after successful therapy. The potential of the novel approach of relating ELlS POT spot-forming cell number and $CD4^+$ T-cell count may improve the possibility of diagnosing active TB and deserves further evaluation.

1756 SAPKOTA, B. R.; RANJIT, C.; NEUPANE, K. D.; MACDONALD, M. **Development and evaluation of a novel multiple-primer PCR amplification refractory mutation system for the rapid detection of mutations conferring rifampicin resistance in codon 425 of the *rpoB* gene of *Mycobacterium leprae*.** *Journal of Medical Microbiology* (2008) **57** (2) 179-184 Reading, UK; Society for General Microbiology [En, 21 ref.] Mycobacterial Research Laboratory, Anandaban Hospital, Kathmandu, Nepal. Email: immuno@tlmnepal.org

Rifampicin-resistant *Mycobacterium leprae* is regularly reported and drug resistance is a major threat for the elimination of leprosy. There is an urgent need for a simple method that can detect rifampicin resistance in clinical isolates. This study developed a multiple-primer PCR amplification refractory mutation system, a

simple, reliable and economical method for clinical specimens that allowed the rapid detection of mutations in the nucleotides of the codon for Ser425 of the *M. leprae rpoB* gene, mutation of which to Leu, Met or 'Phe is associated with rifampicin resistance. The approach involved a multiple-primer PCR in which both mutant specific and normal sets of primers were included in the reaction. The mutant-specific primer was complementary to the corresponding sequence of the wild-type gene except for one additional deliberate mismatch at the fourth nucleotide from the 3' -OH terminus. A single mismatch has little influence on the yield of PCR products, but if there are two mismatches as a result of mutation at the position being tested, the mutant-specific primer will not function in PCR under appropriate conditions, leading to no yield of PCR product from the mutant allele. The assay was evaluated successfully using a panel of plasmids and *M. leprae* reference strains carrying the wild-type or known *rpoB* mutations. The assay was subsequently applied to *M. leprae* DNA extracts from skin biopsies taken from patients. In all biopsy samples, the wild-type allele was detected for Ser425. The PCR results correlated with rifampicin susceptibility, as also measured by the traditional in *vivo* mouse footpad technique.

1757 MAYOSI, B. M.; WIYSONGE, C. S.; NTSEKHE, M.; GUMEDZE, F.; VOLMINK, J. A.; MAARTENS, G.; AlE, A; THOMAS, B. M.; THOMAS, K M.; AWOTEDU, A. A.; THEMBELA, B.; MNTLA, P.; MARITZ, F.; BLACKETT, K. N.; NKOUONLACK, D. C.; BURCH, V. C.; REBE, K.; PARISH, A.; SLIWA, K.; VEZI, B. Z.; ALAM, N.; BROWN, B. G.; GOULD, T.; VISSER, T.; MAGULA, N. P. (ET AL) **Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa.** *SAMJ - South African Medical Journal* (2008) **98** (1) 3640 Pretoria, South Africa; SAMA Health and Medical Publishing Group [En, 17 ref.] Department of Medicine, University of Cape Town, Cape Town, South Africa. Email: bonganimayosi@uct.ac.za

Objective: To determine the mortality rate and its predictors in patients with a presumptive diagnosis of tuberculous pericarditis in sub-Saharan Africa. Design: Between 1 March 2004 and 31 October 2004, we enrolled 185 consecutive patients with presumed tuberculous pericarditis from 15 referral hospitals in Cameroon, Nigeria, and South Africa, and observed them during the 6-month course of antituberculosis treatment for the major outcome of mortality. This was an observational study, with the diagnosis and management of each patient left at the discretion of the attending physician. Using Cox regression, we have assessed the effect of clinical and therapeutic characteristics (recorded at baseline) on mortality during follow-up. Results: We obtained the vital status of 174 (94%) patients (median age 33; range 14-87 years). The overall mortality rate was 26%. Mortality was higher in patients who had clinical features of HIV infection than in those who did not (40% v. 17%, p=0.001). Independent predictors of death during followup were: (i) a proven non-tuberculosis final diagnosis (hazard ratio [HR] 5.35, 95% confidence interval (CI) 1.76 to 16.25), (ii) the presence of clinical signs of HIV infection (HR 2.28, CI 1.14-4.56), (iii) coexistent pulmonary tuberculosis (HR 2.33, CI 1.20-4.54), and (iv) older age (HR 1.02, CI 1.01-1.05). There was also a trend towards an increase in death rate in patients with haemodynamic instability (HR 1.80, CI 0.90-3.58) and a decrease in those who underwent pericardiocentesis (HR 0.34, CI 0.10-1.19). Conclusion: A presumptive diagnosis of tuberculous pericarditis is associated with a high mortality in sub-Saharan Africans. Attention to rapid aetiological diagnosis of

pericardial effusion and treatment of concomitant HIV infection may reduce the high mortality associated with the disease.

2093 ARCE-MENDOZA, A.; RODRIGUEZ-DElTA, J.; SALINAS-CARMONA, M. C.; ROSAS-TARACO, A. G. **Expression of CD64, CD206, and RAGE in adherent cells of diabetic patients infected with *Mycobacterium tuberculosis*.** *Archives of Medical Research* (2008) **39** (3) 306-311 New York, USA; Elsevier [En, 28 ref.] Universidad Autonoma de Nuevo Leon, Immunology, Gonzalitos #235 Norte, Mitras Centro, Monterrey, Nuevo Leon 64460, Mexico. Email: aya_mayola@yahoo.com

Background. CD64 and CD206 receptors play an important role in the internalization of *Mycobacterium tuberculosis* into macrophages. RAGE, described in diabetes (a predisposing factor for tuberculosis), captures glycosylated proteins. Methods. Four groups of 15 patients with type 2 diabetes mellitus (DM2), pulmonary tuberculosis (PTB), type 2 diabetes and pulmonary tuberculosis (DM2-PTB), and controls (CG) were studied. Blood was obtained and mononuclear cells (MNC) isolated and cultured to obtain adherent cells (AC) and then stimulated with *M. tuberculosis* H37Rv lipids. Expression of CD64, CD206 and RAGE was measured by flow cytometry. Results. In the groups without stimulus, PTB and DM2-PTB expressed greater mean fluorescence intensity (MFI) of CD64 and CD206 compared to CG. DM2-PTB showed a decrease in expression compared to PTB. After lipid stimulation no significant difference between groups occurred. In AC without stimulus, RAGE expression was significantly greater in DM2, PTB and DM2-PTB. When DM2-PTB was compared to PTB, a significant decrease in expression occurred. After lipid stimulation, only DM2 cells showed greater MFI. Conclusions. Diabetes affects expression of the three receptors. PTB cells significantly increase them. Diabetes and tuberculosis infection decrease expression compared to PTB alone. Diabetes did not alter CD64 and CD206 expression in infected patients. RAGE expression increases in patients with PTB as well as in diabetics. This suggests that RAGE could also behave as a receptor for *M. tuberculosis*.

2094 KATOCH, K.; PADAM SINGH; ADHTKARI, T.; BENARA, S. K.; SINGH, H. B.; CHAUHAN, D. S.; SHARMA, V. D.; LAVANIA, M.; SACHAN, AS.; KATOCH. V. M. **Potential of *Mw* as a prophylactic vaccine against pulmonary tuberculosis.** *Vaccine* (2008) **26** (9) 1228-1234 Amsterdam, Netherlands; Elsevier [En, 31 ref.] National JALMA Institute for Leprosy and Other Mycobacterial Diseases (ICMR), Tajganj, Agra, UP 282 001, India. Email: kirankatoch@rediffmail.com, rohinik@sancharnet.in, vishwamohan_katoch@yahoo.co.in

Mycobacterium w (*Mw*), is a cultivable, non-pathogenic mycobacterium and has been tried extensively as an immunomodulator in leprosy. This has been found to be safe and has shown beneficial immunoprophylactic effect in population based, double blind placebo controlled trials in North India. These effects were also observed in the vaccine trials in South India. Keeping in view these beneficial effects and its earlier reported protective effect against tuberculosis in animals, its protective efficacy was evaluated in a rural population of about 28948 people belonging to 272 villages in Ghatampur, Kanpur (India). The population was vaccinated with two doses (1st dose of 1×10^9 heat killed organisms followed 6 months later with a 2nd dose of 5×10^8 organisms) of *Mw* 10-13 years ago originally to investigate its effect against leprosy. The vaccine/placebo was given to healthy

contacts of leprosy patients who had no evidence of suffering from tuberculosis. Incidence and prevalence of pulmonary tuberculosis in the present study was assessed in a blind manner by an active field survey and also retrospectively by history of anti tuberculosis treatment received by the patient in the intervening period (since vaccination), which was also corroborated by scrutinizing the medical records. Diagnosis was confirmed by standard clinical and bacteriological criteria. A total of 69 patients were diagnosed to be suffering from pulmonary tuberculosis during the survey which included 17 new sputum smear positive cases and 52 previously partially treated but still active pulmonary tuberculosis cases. The difference in the new sputum positive cases between the vaccinated (5/17) and placebo groups (12/17) was significant at 5% level of significance for I tailed test (Z>1.64). As 75% (52/69) of the cases had been diagnosed as suffering from pulmonary tuberculosis but had not taken adequate therapy all the cases diagnosed during the intervening period were recorded and re-analysis done. The differences are more significant at 1% level of significance for 1 tail test (Z>2.59) when all cases were analysed as a group. A small proportion 12.85% (total number=3036) of the contacts in the study population had BCG scars. On analysis of results on protection against tuberculosis in this group, BCG did provide protection against tuberculosis (p<0.01). In the placebo group the prevalence of tuberculosis was 1.11 % which reduced to 0.70% for those who received *Mw* vaccine (p<0.01) which further decreased to 0.53% in those who had BCG scars and received *Mw*. These results thus provide evidence suggesting protective efficacy of *Mw* against pulmonary tuberculosis and that *Mw* merits investigation in future prospective immunoprophylactic trials along with other candidates for protection against pulmonary tuberculosis.

2095 JONES, K. D. J.; HESKETH, T.; YUDKIN. J. **ExtensIvely drug-resistant tuberculosis in sub-Saharan Africa: an emerging public-health concern.** *Transactions of the Royal Society of Tropical Medicine and Hygiene* (2008) **102** (3) 219-224 Oxford, UK; Elsevier [En, many ref.] UCL Centre for International Health and Development, The Institute of Child Health, London, UK. Email: kelseyjones@gmail.com

The extensively drug-resistant tuberculosis (XDR-TB) categorisation has been developed as a phenotypic description of those TB strains that are resistant to most conventional anti-TB drugs. While widely accepted to have significant incidence in those areas, such as Eastern Europe, that have high levels of multi drug resistance, recent reports have described a cluster of XDR-TB cases in the KwaZulu-Natal province of South Africa. With very high case fatality rates in this setting and a paucity of potential treatment options, concerns have grown about the possibility of an outbreak of highly lethal TB occurring in areas where TB prevalence, generally, is at its highest. In this article, we review previously documented case series of XDR- TB, and examine questions around the likelihood of rapid XDR-TB expansion in sub-Saharan Africa. We analyse how current TB control measures in the area might cope with such a challenge, and identify new areas for focus within the research and development community.

2096 RASHEED, M. U.; GETACHEW BELAY **Nocardiosis in HIV seropositive clinically suspected pulmonary tuberculosis patients.** *Tropical Doctor* (2008) **38** (1) 34-35 London, UK; Royal Society of Medicine Press Ltd [En, 10 ref.] School of Medical Laboratory Technology, Medical

Faculty, Jimma University, POB 378, Jimma, Ethiopia. Email: hmzrshd@yahoo.co.in

To determine the frequency of nocardiosis in HIV-positive individuals clinically suspected of having tuberculosis (TB), 140 sputum samples were collected and processed by Gram staining, modified Ziehl-Neelsen staining and by culture on Lowenstein Jensen medium. Of the 140 patients recruited from Jimma, Ethiopia between May and July 2005, four (2.85%) patients were positive for *Nocardia* sp. by microscopy and 5 (3.6%) had positive culture for *Nocardia asteroides*. In areas where HIV-associated TB is common, some patients diagnosed as smear-negative pulmonary TB will actually have nocardiosis. Clinicians should be aware of this entity in HIV/immunocompromised patients with respiratory infections who fail to respond to antituberculous treatment.

2097 MATEE, M.; MTEI. L.; LOUNASVAARA, T.; WIELAND-ALTER, W.; WADDELL, R; LYIMO, J.; BAKARI, M.; PALLANGYO, K.; REYN, C. F. VON **Sputum microscopy for the diagnosis of HIV - associated pulmonary tuberculosis in Tanzania.** *BMC Public Health* (2008) **8** (68) (21 February 2008) London, UK; BioMed Central Ltd [En, 27 reL] Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania. Email: mmateerrr@yahoo.com. Indefomiro@yahoo.com, tarja.lounasvaara@luukku.com, Wendy.F. Wieland-Alter@Dartmouth.EDU, Richard.D.Wad-dell@Dartmouth.EDU, Jlyimo@yahoo.com, mbakari@muhas.ac.tz, Kpallangyo@muhas.ac.tz, C.Fordham. von.Reyn@Dartmouth.EDU

Background: In many resource poor settings only sputum microscopy is employed for the diagnosis of HIV-associated pulmonary tuberculosis; sputum culture may not be available. Methods: We determined the diagnostic accuracy of sputum microscopy for active case finding of HIV -associated pulmonary tuberculosis using TB culture as the reference standard. Results: 2216 potential subjects screened for a TB vaccine trial submitted 9454 expectorated sputum specimens: 212 (2.2%) were sputum culture positive for *Mycobacterium tuberculosis* (MTB), 31 (0.3%) for non-tuberculous mycobacteria, and 79 (0.8%) were contaminated. The overall sensitivity of sputum microscopy was 61.8% (131/212) and specificity 99.7% (9108/9132). Sputum microscopy sensitivity varied from 22.6% in specimens with <20 colony forming units (CFU)/specimen to 94.2% in patients with >100 CFU/ specimen plus confluent growth. The incremental diagnostic value for sputum microscopy was 92.1 %, 1.8% and 7.1 % for the first, second and third specimens, respectively. The positive predictive value and negative predictive values for sputum microscopy were 84.5% and 99.1 %, respectively. The likelihood ratio (LR) of a positive sputum microscopy was 235.1 (95% CI 155.8-354.8), while the LR of a negative test was 0.38 (95 CI 0.32-0.45). The 212 positive sputum cultures for MTB represented 103 patients; sputum microscopy was positive for 57 (55.3%) of 103 patients. Conclusion: Sputum microscopy on 3 expectorated sputum specimens will only detect 55% of culture positive HIV-infected patients in active screening for pulmonary tuberculosis. Sensitivity is higher in patients with greater numbers of CFUs in the sputum. Culture is required for active case finding of HIV-associated pulmonary tuberculosis.

2098 MOET, F. J.; PAHAN, D.; OSKAM, L.; RICHARDUS, J. H. **Effectiveness of single dose rifampicin in preventing leprosy in close contacts of patients with newly diagnosed leprosy: cluster randomised controlled trial.** *BMJ* (2008) **336** (7647)

761-764 London, UK; BMJ Publishing Group [En, II ref.] Depat1ment of Public Health, Erasmus MC, University Medical Center Rotterdam, PO Box 2040, 3000 CA Rotterdam, Netherlands. Email: j.richardus@erasmusmc.nl

Objective: To determine the effectiveness of chemoprophylaxis using a single dose of rifampicin to prevent leprosy in close contacts. Design: Single centre, double blind, cluster randomised, placebo controlled trial. Setting; Leprosy control programme in two districts of northwest Bangladesh with a population of more than four million. Participants: 28 092 close contacts of 1037 patients with newly diagnosed leprosy. 21 711 contacts fulfilled the study requirements. Interventions: A single dose of rifampicin or placebo given to close contacts in the second month of starting the index patient's treatment, with follow-up for four years. Main outcome measure: Development of clinical leprosy. Results: 18869 of the 21 711 contacts (86.9%) were followed-up at four years. Ninety one of 9452 contacts in the placebo group and 59 of 9417 in the rifampicin group had developed leprosy. The overall reduction in incidence of leprosy using a single dose of rifampicin in the first two years was 57% (95% confidence interval 33% to 72%). The groups did not differ between two and four years. The overall number needed to treat (NNT) to prevent a single case of leprosy among contacts was 297 (95% confidence interval 176 to 537). Differences were found between subgroups at two years, both in reduction of incidence and in NNT. Conclusion: A single dose of rifampicin given to contacts of patients with newly diagnosed leprosy is effective at preventing the development of clinical leprosy at two years. The effect was maintained, but no difference was seen between the placebo and rifampicin groups beyond two years.

2099 LI YUE; WANG YOUGAN **Smooth bootstrap methods for analysis of longitudinal data.** *Statistics in Medicine* (2008) **27** (7) 937-953 Chichester, UK; John Wiley & Sons [En, 21 ref.] Lilly-Singapore Centre for Drug Discovery, I Science Park Road #04-01, The Capricorn, Singapore 117528, Singapore. Email: Yougan.Wang@csiro.au

In analysis of longitudinal data, the variance matrix of the parameter estimates is usually estimated by the 'sandwich' method, in which the variance for each subject is estimated by its residual products. We propose smooth bootstrap methods by perturbing the estimating functions to obtain 'bootstrapped' realizations of the parameter estimates for statistical inference. Our extensive simulation studies indicate that the variance estimators by our proposed methods can not only correct the bias of the sandwich estimator but also improve the confidence interval coverage. We applied the proposed method to a data set from a clinical trial of antibiotics for leprosy.

2100 SAKHA, K.; BEHBAHAN, A. G. **Immunogenicity of neonatal BCG vaccination in children entering primary school.** *Pakistan Journal of Biological Sciences* (2008) **11** (6) 930-933 Faisalabad, Pakistan; ANSInet, Asian Network for Scientific Information [En, 17 ref.] Department of Pediatrics, Tabriz Children's Hospital, Tabriz University of Medical Sciences, Sheshgelan St., Tabriz, Iran.

This study has been designed to evaluate the immunogenicity of neonatal BCG-vaccination in children at the age of 7 to 8 years, by skin test using Purified Protein Derivative (PPD), as BCG vaccination at birth is a part of routine program of immunization in our country, Iran; we decided to study its efficacy and also tried to determine if there is any correlation between PPD-test results and BCG scar size. This is a comparative study on

150 children (94 males and 56 females) at the age of 7 to 8 years, who possess neonatal-BCG scar. They were chosen from several primary schools in Tabriz-Iran, by simple random sampling and tested with 0.1 mL of 5-unit-PPD solution (a product of Iran Institute of Razi); then observations recorded. The average diameter of BCG scars were 7.03 mm in girls, 5.45 mm in boys and 6.05 for all. The diameter of induration area resulted from PPD-test after 72 h was less than 5 mm in 95.33% and 5-9 mm in 4.66% of studied children; there was no case with induration area of 10 mm or more at all. Every child who developed an induration area of 5 mm or more by PPD test, had a BCG scar with the diameter of 5 mm or more. There was a statistically meaningful direct correlation between sizes of neonatal-BCG scar and diameter of induration area after PPD-test (r=0.21 and p=0.008). This study shows that reactivity to PPD test (and probably immunity against tuberculosis) decreases as age increases; therefore it seems to be necessary to repeat BCG-vaccination in children at the age of entering primary school.

2101 UMUBYEYI, A; RIGOUTS, L.; SHAMPUTA, I. C.; DEDISTE, A.; STRUELENS, M.; PORTAELS, F. **Low levels of second-line drug resistance among multidrug-resistant *Mycobacterium tuberculosis* isolates from Rwanda.** *International Journal of Infectious Diseases* (2008) **12** (2) 152-156 Oxford, UK; Elsevier [En, 19 ref.] Department of Microbiology, CHU St Pierre, Brussels, Belgium. Email: alainenyaruhirira@hotmail.com

Background: Multidrug-resistant tuberculosis (MDR-TB) has become a therapeutic problem in many parts of the world, necessitating the inclusion of second-line anti-tuberculosis drugs in specific treatment regimens. Methods: We studied the susceptibility of 69 MDR *Mycobacterium tuberculosis* isolates from Rwanda to second-line drugs by the BACTEC 460 method. Results: The results showed that 62 (89.9%) were resistant to rifabutin while a low rate (4.3%) of resistance was registered for ofloxacin; there was one case (1.4%) of resistance each for *para*-aminosalicylic acid, kanamycin, ethionamide, and clarithromycin. Conclusions: This information is important for devising an appropriate treatment regimen for MDR- TB patients in order to stop the spread of MDR strains and contain the acquisition of additional drug resistance in Rwanda.

2102 SINGH, A. V.; SINGH, S. V.; MAKHARIA, G. K.; SINGH, P. K.; SOHAL, J. S. **Presence and characterization of *Mycobacterium avium* subspecies *paratuberculosis* from clinical and suspected cases of Crohn's disease and in the healthy human population in India.** *International Journal of Infectious Diseases* (2008) **12** (2) 190-197 Oxford, UK; Elsevier [En, 56 ref.] Veterinary Microbiology Laboratory, Animal Health Division, Central Institute for Research on Goats, Makhdoom, Farah, Mathura (UP) 281 122, India. Email: shoorvir.singh@gmail.com

Objectives: To investigate and characterize *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in patients with Crohn's disease, attendants of animals with suspected infection, and healthy human, using multiple diagnostic tests, Methods: A total of 119 samples (35 stool, 76 serum, three blood clots, and five biopsies) were collected from five patients with Crohn's disease, eight attendants of animals with lohne's disease, and 93 apparently normal control subjects (Agra region) from North India. Samples were screened for the presence of MAP by smear examination, culture of stool, blood clot and biopsies, and ELISA. Colonies

obtained by culture were further characterized using polymerase chain reaction (PCR) with IS900 MAP-specific primers. Results: Using all diagnostic modalities, MAP and/or MAP antibodies were identified in 100% (5/5) of subjects with Crohn's disease; 75.0% (6/8) of attendants of MAP infected animals were positive and 38.0% (27/71) of apparently normal controls were also positive. Most sensitive test was ELISA (100%, 5/5), followed by culture (80.0%, 4/5), and acid-fast staining. Ziehl-Neelsen staining was positive in 37.5% (3/8) of subjects with active animal husbandry practices. In 71 serum samples from control subjects, seroprevalence of MAP was 38.0% using indigenous protoplasmic antigens (PPA) and 36.6% using commercial PPA. Of the serum samples from the Crohn's disease patients, 100% (5/5) were positive by ELISA using indigenous PPA and 40.0% (2/5) were positive by ELISA using commercial PPA. IS900 PCR was used to characterize tiny colonies of MAP that grew extremely slowly on Herrold's egg yolk medium, and of 15 (42.8%) cultures, 14 (93.3%) were typed as MAP. Conclusions: Paper documented the presence of MAP in all patients with Crohn's disease, in some animal attendants who had the history of working with goat herds infected with lohne's disease and in few normal healthy individuals. Presence of Ziehl Neelsen positive MAP. In the stool of attendants working with MAP-infected animals was unique to humans. ELISA based on antigens derived from indigenous MAP 'bison type' genotype of goat origin was most sensitive modality for screening Crohn's disease patients.

2103 MWINGA, A.; MWANANYAMBE, N.; KANENE, C.; BULTERYS, M.; PHIRI, C.; KAPATA, N.; MUKONKA, V.; NADOL, P.; PATEL, M.; NAKASHIMA, **A. Provider-initiated HIV testing and counseling of TB patients - Livingstone District, Zambia, September 2004 December 2006.** *Morbidity and Morrality Weekly Report* (2008) **57** (11) 285-289 Atlanta, USA; Epidemiology Program Office, Centers for Disease Control and Prevention (CDC) [En, 9 ref.] CDC Global AIDS Program Zambia, Zambia.

This report summarizes the results of a provider-initiated HIV testing and counselling (PITC) pilot study conducted in Livingstone District, Southern Province of Zambia, during September 2004-December 2006. Of the 4148 tuberculosis cases recruited from 3 clinics, 2545 (61%) were counselled and 2072 (50%) were tested for HIV. 1497 (72%) tested positive for HIV. These findings demonstrate the practicality and acceptance of PITC and HIV rapid testing, and support the need to expand this programme to tuberculosis clinical settings in Zambia and other countries with high rates of tuberculosis and HIV.

2104 OLOYA, J.; OPUDA-ASIBO, J.; KAZWALA, R.; DEMELASH, A. B.; SKJERVE, E.; LUND, A.; JOHANSEN, T. B.; DJONNE, B. **Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda.** *Epidemiology and Infection* (2008) **136** (5) 636-643 Cambridge, UK; Cambridge University Press [En) Department of Veterinary Public Health and Preventive Medicine, Makerere University, Kampala, Uganda. Email: Eystein.Skjerve@veths.no

Mycobacteria from lymph node biopsies of patients with cervicallymphadenitis reporting for tuberculosis treatment in Matany and Moroto Hospitals in the transhumant areas of Karamoja, Uganda were isolated and characterized. The AccuProbe® culture identification kits for *Mycobacterium tuberculosis* complex (MTC), *M. avium* complex (MAC) and *M. avium* were

used to identify the isolates. Spoligotyping, IS901 PCR and IS1311 and IS1245 restriction fragment length polymorphism (RFLP) were used to characterize the isolates. Of the 43 biopsies, ten *M. avium*, seven *M. tuberculosis*, three *M. bovis*, and two *M. intracellulare* were isolated. Two isolates could not be identified with AccuProbe® and from 19 samples no mycobacteria could be isolated. Three isolates with the Beijing spoligotype were identified from the seven *M. tuberculosis* isolates. The spoligopatterns of the *M. bovis* isolates had previously been detected in cattle in Uganda. Isolation of members of the MAC group reflects the complex interaction between the transhumant communities, water sources and their cattle. None of the *M. avium* isolates harboured IS901, and all showed several bands on IS1311 and IS1245 RFLP, in accordance with *M. avium* subsp. *hominissuis*. Composite dendrograms of ISI311 and ISI245 RFLP showed that the isolates were similar and identical patterns were found. The isolation of *M. bovis* confirms the human infection with zoonotic mycobacteria in areas where consumption of raw milk and meat is routine. Isolation of environmental mycobacteria also confirms their increasing role in human disease and the occupational risk of infection in the transhumant ecosystem in the absence of safe drinking water and environmental contamination.

2105 TALAY, F.; KUMBETLI, S.; ALTIN. S. **Factors associated with treatment success for tuberculosis patients: a single center's experience in Turkey.** *Japanese Journal of Infectious Diseases* (2008) **61** (1) 25-30 Tokyo, Japan; National Institute of Infectious Diseases (NIID) [En, 10 ref.] Department of Chest Diseases, Izzet Baysal Faculty of Medicine, Abant Izzet Baysal University, 14280 Bolu, Turkey. Email: ftalay2000@yahoo.com

We aimed to evaluate the treatment outcome of pulmonary tuberculosis patients and factors affecting treatment outcomes. We analysed the records of 586 pulmonary tuberculosis patients who were older than 15 years followed between January 1999 and December 2004 at the Istanbul Eyup Tuberculosis Dispensary, Istanbul, Turkey. Of these patients, 76.1 % were smear-positive for tuberculosis and 23.9% were smear-negative for tuberculosis. The treatment outcomes of all patients analysed were as follows: treatment success 91.7%, defaulted treatment 5.1 %, died 2.4%, failure 0.3%, and transferred out 0.5%. The treatment outcomes of smear positive pulmonary tuberculosis patients were as follows: cured 77.1 %, treatment completed 13.5%, defaulted treatment 5.4%, died 2.9%, failure 0.4%, and transferred out 0.7%. In multivariate regression analysis, risk factors for non-successful treatment outcome were determined to be re-treatment patients, patients older than 46 years of age, and the presence of rifampicin resistance. We conclude that application of Directly Observed Therapy may increase treatment success in all patients, especially patients who have risk factors for a low treatment success rate.

2106 OZSAHIN, S. L.; TURGUT, B.; NUR, N.; DOGAN, O. T.; ERSELCAN, T.; BERK, S. **Validity of the CA125 level in the differential diagnosis of pulmonary tuberculosis.** *Japanese Journal of Infectious Diseases* (2008) **61** (1) 68-69 Tokyo, Japan; National Institute of Infectious Diseases (NIID) [En, 14 ref.] Department of Chest Diseases, Faculty of Medicine, Cumhuriyet University, 58140-Sivas, Turkey. Email: naimnur@yahoo.com

The aim of the current study was to determine the possible crucial role of cancer antigen 125 (CA125) in the diagnosis of

pulmonary tuberculosis (PTB). The CA125 levels of study and control groups were statistically compared. In a total of 146 patients that were included in the current study, 30 had active PTB, 37 inactive PTB, 28 community-acquired pneumonia (CAP), 25 pleural or pulmonary malignancies, and 13 patients exacerbation of chronic obstructive pulmonary disease [Turkey]. The mean CAI25 levels in PTB, inactive PTB, CAP, and pleural-pulmonary malignancies were 118.46±248.41, 40.80±50.95, 47.76±60.76, and 57.77±65.59, respectively. For active-inactive discrimination of PTB, with a cut-off level of ≥35 U/ml, the sensitivity, specificity, positive predictive value, and negative predictive value of CAI25 were 63, 59, 56, and 67%, respectively. Increased CA125 levels were detected in active PTB in the current results. The current results also show that high level CA125 should be reconsidered in the prediagnosis and/or discrimination of active and inactive PTB patients.

2107 JOB, C. K.; JAYAKUMAR, J.; KEARNEY, M.; GILLIS. T. P **Transmission of leprosy: a study of skin and nasal secretions of household contacts of leprosy patients using PCR.** *American Journal of Tropical Medicine and Hygiene* (2008) **78** (3) 518-521 Northbrook, USA; American Society of Tropical Medicine and Hygiene [En, 23 ref.] St. Thomas Hospital and Leprosy Centre, Chettupattu, India. Email: tgillis@lsu.edu

It is generally held that dissemination of *Mycobacterium leprae* is from nasal mucosa and not through the skin of infected patients. In this study, we evaluated *M. leprae* in the unbroken skin and nasal secretions of multibacillary (MB) leprosy patients from India and their contacts. Specimens were examined by direct microscopy and polymerase chain reaction (PCR) for *M. leprae* DNA. Results showed that 60% of untreated MB leprosy patients examined histologically had acid-fast bacilli in the keratin layer. By PCR studies, it was found that 80% of the patients had *M. leprae* DNA in skin washings and 60% had *M. leprae* DNA on swabs obtained from the nasal mucosa. Ninety-three contacts of the untreated MB cases were also tested for exposure to *M. leprae* by analysing skin washings and nasal secretions by PCR. PCR analysis showed significant skin (17% positive) and nasal mucosal (4%) exposure in contacts before instituting treatment of the index cases. After 2 months of treating the index cases, all contacts tested were negative for *M. leprae* DNA. These data suggested that both skin and nasal epithelia of untreated MB leprosy patients contribute to the shedding of *M. leprae* into the environment and contacts of untreated MB cases are at risk for contact with *M. leprae* through both the nasal mucosa and exposed surfaces of their skin.

2108 JYOTHI, P.; RIYAZ NAJEEBA; NANDAKUMAR, G.; BINITHA, M. P. **A study of oxidative stress in paucibacillary and multibacillary leprosy.** *Indian Journal of Dermatology, Venereology & Leprology* (2008) **74** (1) 80 Mumbai, India; Medknow Publications [En, 12 ref.] CHC, Iriveri, Kannur, Kerala, India. Email: drjyothip02@yahoo.com

Background: The study and assessment of oxidative stress plays a significant role in the arena of leprosy treatment. Once the presence of oxidative stress is proved, antioxidant supplements can be provided to reduce tissue injury and deformity. Aim: To study oxidative stress in paucibacillary (PB) and multibacillary (MB) leprosy and to compare it with that in a control group. Methods: Fifty-eight untreated leprosy patients (23 PB and 35 MB cases) were studied and compared with 58 healthy controls. Superoxide dismutase (SOD) level as a measure of antioxidant status; malond-

ialdehyde (MDA) level, an indicator of lipid peroxidation; and MDA/SOD ratio, an index of oxidative stress were estimated in the serum. Results: The SOD level was decreased in leprosy patients, especially in MB leprosy. The MDA level was increased in PB and MB leprosy. The MDA/SOD ratio was significantly elevated in MB patients. There was a steady increase in this ratio along the spectrum from tuberculoid to lepromatous leprosy (LL). Conclusion: There is increased oxidative stress in MB leprosy, especially in LL. This warrants antioxidant supplements to prevent tissue injury.

2109 VIANA-NIERO, C.; LIMA, K. V. B.; LOPES, M. L.; RABELLO, M. C. DA S.; MARSOLA, L. R.; BRILHANTE, V. C. R.; DURHAM, AM.; LEAO, S. C. **Molecular characterization of *Mycobacterium massiliense* and *Mycobacterium bolletii* in isolates collected from outbreaks of infections after laparoscopic surgeries and cosmetic procedures.** *Journal of Clinical Microbiology* (2008) **46** (3) 850-855 Washington, USA; American Society for Microbiology (ASM) [En, 27 ref.] Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de Sao Paulo, Rua Botucatu, 862, 3° andar, Sao Paulo 04023-062, Brazil. Email: sylvia.leao@unifesp.br

An outbreak of infections affecting 311 patients who had undergone different invasive procedures occurred in 2004 and 2005 in the city of Belém, in the northern region of Brazil. Sixty-seven isolates were studied; 58 were from patients who had undergone laparoscopic surgeries, 1 was from a patient with a postinjection abscess, and 8 were from patients who had undergone mesotherapy. All isolates were rapidly growing nonpigmented mycobacteria and presented a pattern by PCR-restriction enzyme analysis of the *hsp65* gene with BstEII of bands of 235 and 210 bp and with HaeIII of bands of 200, 70, 60, and 50 bp, which is common to *Mycobacterium abscessus* type 2, *Mycobacterium balletii*, and *Mycobacterium massiliense*. *hsp65* and *rpoB* gene sequencing of a subset of 20 isolates was used to discriminate between these three species. *hsp65* and *rpoB* sequences chosen at random from 11 of the 58 isolates from surgical patients and the postinjection abscess isolate presented the highest degrees of similarity with the corresponding sequences of *M. massiliense*. In the same way, the eight mesotherapy isolates were identified as *M. bolletii*. Molecular typing by pulsed-field gel electrophoresis (PFGE) grouped all 58 surgical isolates, while the meso therapy isolates presented three different PFGE patterns and the postinjection abscess isolate showed a unique PFGE pattern. In conclusion, molecular techniques for identification and typing were essential for the discrimination of two concomitant outbreaks and one case, the postinjection abscess, not related to either outbreak, all of which were originally attributed to a single strain of *M. abscessus*.

2110 RIE, A. VAN; FITZGERALD, D.; KABUYA, G.; DEUN, A. VAN; TABALA, M.; JARRET, N.; BEHETS, F.; BAHATI, E. **Sputum smear microscopy: evaluation of impact of training, microscope distribution, and use of external quality assessment guidelines for resource-poor settings.** *Joumal of Clinical Microbiology* (2008) **46** (3) 897-901 Washington, USA; American Society for Microbiology (ASM) [En, 11 ref.] Department of Epidemiology, 2104 McGavran Hall, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7435, USA. Email: vanrie@email.unc.edu

Sputum smear microscopy is the main and often only laboratory technique used for the diagnosis of tuberculosis in resourcepoor

countries, making quality assurance (QA) of mear microscopy an important activity. We evaluated the effects of as-day refresher training course for laboratory technicians and the distribution of new microscopes on the quality of smear microscopy in 13 primary health care laboratories in Kinshasa, Democratic Republic of Congo. The 2002 external QA guidelines for acid-fast bacillus smear microscopy were implemented, and blinded rechecking of the slides was performed before and 9 months after the training course and microscope distribution. We observed that the on-site checklist was highly time-consuming but could be tailored to capture frequent problems. Random blinded rechecking by the lot QA system method decreased the number of slides to be reviewed. Most laboratories needed further investigation for possible unacceptable performance, even according to the least-stringent interpretation. We conclude that the 2002 external QA guidelines are feasible for implementation in resource-poor settings, that the efficiency of external QA can be increased by selecting sample size parameyrs and interpretation criteria that take into account the local forking conditions, and that greater attention should be paid to t provision of timely feedback and correction of the causes of su tandard performance at poorly performing laboratories.