## 225. Tuberculosis: from bench to bedside

**P1809**

**$T_{reg}$ cells are expanded among bronchoalveolar lymphocytes in healthy tuberculosis contacts with positive interferon-γ release assay responses**

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**Background:** A positive Interferon-γ release assay (IGRA+) after exposure to tuberculosis indicates a systemic immune response to *Mycobacterium tuberculosis* (MTB). However, most people will remain IGRA-negative (IGRA-) after exposure. In mice, regulatory T-lymphocytes ($T_{reg}$) can deter the pulmonary clearance in early infection, possibly preventing a systemic immune response.
**Objective:** To study the local immunity in TB-exposed health care workers without active disease.
**Methods:** FACS analysis on bronchoalveolar lavage (BAL) cells including $T_{reg}$ cells (CD4+CD25+CD127-) and activated macrophages (HLA-DR+). Questionnaire for demographic, and health-related data. Blood IGRA.
**Results:** Demographic and epidemiological parameters were not statistically different. Of 10 IGRA- and 12 IGRA+ subjects, the BAL of the latter group showed a

higher frequency of $T_{reg}$ cells (median 2,5% [range 1.4-4.4%] vs. 0.7% [0.2-1.3%]; p=0.0001 for Mann-Whitney test). In contrast, the frequency of $T_{reg}$ cells in among peripheral blood monocytes (PBMC) was similar in both groups (22% [10-38%] vs. 21% [9-27%]; p=0.87). Accordingly, activated macrophages in the BAL were found more frequently in IGRA-positive than in IGRA negative subjects (median 89% [range 77-100] vs. 95% [85-100]; p=0.04), while there was no difference in blood (91% [82-94%] vs. 86% [74-96%]; p=0.34).
**Conclusion:** In healthy contacts of tuberculosis patients, a positive IGRA response is associated with expansion of $T_{reg}$ cells and activated macrophages in the BAL but not in peripheral blood. The role of these cell populations for the prevention of systemic immune responses to MTB needs to be further evaluated.

**P1810**
**Phagocytic activity and pattern of Fcγ and complement receptors on blood monocytes in pulmonary tuberculosis**
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Tuberculosis (TB) remains a worldwide public health problem. Mycobacterium tuberculosis (Mtb) infects approximately one-third of the population. Nearly 8 million active TB cases and 2 million deaths from TB are recorded each year. Majority of Mtb infected individuals control the infection by mounting cell-mediated immune responses but we don't know why only 5–10% of infected cases develop active TB. Because of CD14, receptors for Fc fragment of IgG (FcγR) and complement (CR) on phagocytes play key role in immune response to Mtb, we have evaluated the expression of receptors FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16) and CR1 (CD35), CR3 (CD11b), CR4 (CD11c) and phagocytic activity of CD14+ monocytes in peripheral blood of 20 patients with TB and 20 healthy volunteers using the flow cytometry. Our study revealed significantly increased presence of receptors CD14+CD32+, CD14+CD35+, and CD14+CD11b+ on monocytes in TB patients than in controls. Analysis of phenotypes both FcγR and CR receptors on CD14+ monocytes revealed significantly increased presence of monocytes CD14+CD64-CD11b+ and CD14+CD16-CD11b+ and insignificant CD14+CD32-CD11b+. Phagocytic activity of blood monocytes in TB patients was significantly higher than in healthy individuals. In summary, increased frequency of CD14+ monocytes with FcγRII, CR1, and CR3 may explain increased adherence and phagocytosis of Mtb with following development of TB. Therefore, the receptors FcγRII and CR1, CR3 could be considered as drug target for treatment of TB.

**P1811**
**The impact of absolute CD4+ and CD8+ T-cell counts on IGRA test results in HIV/AIDS patients**
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**Background:** Identification and treatment of LTBI in HIV-positive individuals is one of the main recommendations of WHO. New IFN-γ release assays (IGRAs) are based on MTB-specific T-cell responses which are impaired in HIV/AIDS pts due to decreased numbers and function of CD4+/CD8+ T cells.
**Aim:** To study the influence of CD4+ and CD8+ T-cell counts on IGRA results in HIV/AIDS pts.
**Matherials and methods:** QuantiFERON-TB Gold In-Tube (QFT-GIT) (Cellestis, Australia) and T-SPOT.TB (SPOT) (Oxford Immunotec, UK) tests were performed according to manufacturers' instructions. CD4+/CD8+ T-cell counts were determined by flowcytometry (BD, FACSCanto II). Spearman correlation and t-test were used for statistical analysis. All pts provided written informed consent.
**Results:** From January 2010 to January 2011, 105 (62% males) (18-66 yrs) HIV-infected individuals in different stages of progression were recruited. The mean CD4+ and CD8+ count was 448 cells/μl (2–1731) and 1000 cells/μl (74-3871) respectively. 24/105 pts were positive in both IGRAs. In all 24 pts with positive IGRA results MTB-specific and Mitogen-induced IFN-γ production did not correlate with absolute CD4+/CD8+ T-cell counts ($p>0.05$). Mean values of CD4+/CD8+ T cells in pts with indeterminate results were insignificantly lower than in pts with negative and positive results in both IGRAs ($p>0.05$).
**Conclusions:** Our data demonstrate that TB antigen-specific immune responses can be detected in HIV/AIDS pts with low CD4+ and CD8+ T-cell counts using IGRAs. Both QFT-GIT and SPOT assays are useful immunodiagnostic tools in immunocompromised HIV pts.
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**P1812**
**Cytokines in pathogenesis of pulmonary tuberculosis**
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**Introduction:** The present work is devoted to relative research of some cytokines (IL-1β, IL-8, IL-2, TNF-α) at the tuberculosis patients with the subsequent evaluation of their role in pathogenesis of various forms of pulmonary tuberculosis.
**Aim:** The aim of the study is to assess the severity of immune system disorders in patients with progressive forms of tuberculosis
**Materials and methods:** The clinical and immunological inspection is executed at 193 patients with active pulmonary tuberculosis.
**Results:** At infiltrative tuberculosis the high level of production IL-1β and IL-8 was combined with the maximal stimulation index of IL-1β and TNF-α, but low stimulation index of IL-8. Production of IL-2 exceeded the patients with more serious forms of a tuberculosis - disseminated and caseous pneumonia. Fibro-cavernousis tuberculosis was characterized by more intensive production of IL-2, low serumal contents of IL-1β, TNF-α and lower activity of IL-8. The level of inflammatory cytokines production (IL-1β and TNF-α), alongside with a low index of their stimulation is characteristic for a disseminated tuberculosis, that testifies to a state of hyper activation of immunocompetent cells. In caseous pneumonia, alongside with the maximal reduced of IL-1β, TNF-α and IL-2, the highest index of stimulation IL-1β and TNF-α was marked.
**Conclusion:** The received data testify to the highest degree of an activation of cells of cytophagous in patients with infiltrative tuberculosis, and their expressed functional incompetence in caseous pneumonia. The down stroke of production not only IL-2, but also IL-1β alongside with a high level of IL-8, corresponds to the extremely serious state of the patients.

**P1813**
**Lethal tuberculosis in a previously healthy adult with IL-12 receptor deficiency**
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**Introduction:** Most patients with disseminated tuberculosis (TB) have no known underlying immunodeficiency. Here, we report the first case of disseminated TB in an adult patient with underlying IL-12Rβ1 deficiency.
**Case:** A 33 year old man was admitted in hospital due to fever, generalized lymphadenopathy, and hepatosplenomegaly. He had a history of anti-TB treatment in the previous 3 years. Despite normal chest X-ray, sputum smear was positive for Acid-fast bacilli (AFB) and PCR was positive for Mycobacterium tuberculosis complex. Drug susceptibility test revealed multi-drug resistance (MDR) to INH and RIF. Standard MDR-TB treatment was initiated. Evaluation of immunologic and genetic status of patient revealed IL-12Rβ1 deficiency.
Despite initial response to treatment, the patient developed profuse diarrhea. In colonoscopy, polypoid lesions were observed which were full of AFB. Linezolid and IFN-γ were added to the treatment but the patient passed away due to disseminated TB.
**Conclusion:** IL-12Rβ1 deficiency is a genetic etiology of severe TB in adults. We should consider genetic defects of the IL-12-IFN-γ circuit in adult patients with disseminated TB.

**P1814**
**Genotyping of M. tuberculosis from selected population of Kanpur City, north India**
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**Background:** Genotyping of M. tuberculosis (MTB) is a better tool to study prevalent strains and transmission trends. Spoligotyping relies on polymorphism of direct repeat (DR) sequences of MTB. Isolating DNA directly from sputum smear slides will be convenient and less time consuming. Sequencing rpoB (reverse polymerase B) gene detects rifampicin resistance which act as surrogate marker of MDR TB. In this study, spoligotyping and sequencing of rpoB gene was done from sputum smear positive slides obtained from selected regions of Kanpur, India.
**Material and methods:** A total of 139 sputum smear positive patients were randomly selected from eleven DOTS centers covering contiguous areas of Kanpur region, India from january2009 to august2010. DNA was extracted directly from the Ziehl Neelsen stained sputum smear slides and spoligotyping to detect 43 known spacers in the DR locus of MTB was done. 350 base pair region of rpoB gene was amplified using oligonucleotide primers.
**Results:** Out of 139 samples, spoligotyping was possible in 98 (70.5%). ST100/MANU 1 is the predominant spoligotype (48.9%) in the studied region. The other major spoligotypes were ST523/U (6.2%), ST54/MANU2 (2%) and 26.5% were unique. Spoligotyping was more possible in highly positive (3+/2+) versus less positive (1+/scanty +) slides ($p<0.0001$). rpoB gene sequencing was possible in only 14 (10.1%) samples and out of which rifampicin resistance was present in 3/14 (21%).
**Conclusions:** DNA isolation and spoligotyping was found to be feasible directly from ZN stained smears but rpoB gene sequencing was difficult. Using samples

like sputum smears is time saving, simple and convenient compared with other culture based methods.

**P1815**

**Genetic association studies between the MyD88 adaptor pathway SNPs and the development of tuberculosis**

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**Background:** Tuberculosis (TB) is characterized by the formation of granulomas. Myeloid Differentiation factor (MyD)88 is the common adaptor molecule that communicates the Toll-like receptor (TLR) engagement on the cell surface to intracellular events. TLRs are extracellular receptors that recognize specific molecular patterns found in a broad range of microbial pathogens, triggering inflammatory responses. There is evidence that MyD88-/- animals fail to form granulomas, while animal studies indicate that MyD88 signalling pathway is crucial for the control of *M.Tuberculosis* infection.
**Objectives:** We hypothesize that a defect in MyD88 pathway results in increased susceptibility of *M. tuberculosis* infection through reduced immune response following the recognition of the microorganism by TLR proteins. To this end we analyzed SNPs in the *MyD88* gene.
**Methods:** 93 TB patients with culture-proven or smear-positive microscopy active TB and 92 controls were analyzed for the following SNPs; *MyD88* -938C>A and *MyD88* 1944C>G.
**Results:**. The MyD88-938CA genotype is associated with an increased risk of developing TB with an Odds Ratio (OR) of 2.62 (95% Confidence Intervals [CIs] 1.31-5.22, p=0.006) while the MyD881944CC genotype is associated with an increased risk of developing TB (OR: 4.83 (95% CIs: 1.86-12.52, p=0.001).
**Conclusions:** The results indicate that the MyD88-938CA and the MyD881944CC genotypes may be associated with increased *M.Tuberculosis* infection susceptibility. Since 1/3 of the worlds population has been in contact with *M. tuberculosis* but only 10% of them will develop an active infection, our findings provide important insights regarding tuberculosis development.

**P1816**

**NALP3 and CARD8 genetic polymorphisms and antituberculosis-drugs induced hepatitis**

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**Backgrounds:** Genetic susceptibility to the development of antituberculosis drugs (ATD)-induced hepatitis is poorly understood yet. The NALP3 inflammasomes, which sense danger signals and produce IL-1β, may contribute to the initiation of inflammatory response and ATD-induced hepatitis. We examined if the polymorphisms in NALP3 inflammasome genes (*NLRP3* and *CARD8*) are associated with ATD-induced hepatitis.
**Methods:** We enrolled 80 patients with ATD-induced hepatitis and 238 ATD-tolerant subjects. DNA was isolated from whole blood and genotyped for the single nucleotide polymorphisms (SNPs) in *NLRP3* and *CARD8*. Genotype frequencies of SNPs and haploptyes were compared between patients with ATD-induced hepatitis and ATD-tolerant patients.
**Results:** Of seven SNPs of *NALP3* (rs35829419, rs4612666, rs10754558, rs4353135, rs55646866, rs72655377 and rs10733113), there was no significant association with ATD-induced hepatitis. Analysis of *NALP3* haplotypes found no significant relationship with ATD-induced hepatitis. C10X of CARD8 (rs2043211) was not associated with the risk of ATD-induced hepatitis.
**Conclusions:** These findings indicate that *NALP3* and *CARD8* genetic polymorphisms are not associated with the development of ATD-induced hepatitis in Korean population, and suggest that NALP3 inflammasome do not play important roles in the pathogenesis of ATD-induced hepatitis.

**P1817**

**Mutations of the gyrA gene of mycobacterium tuberculosis leading to XDR tuberculosis in Kyrgyz Republic**

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In the treatment of multidrug-resistant tuberculosis (MDR) the second line drugs are prescribed and among them the most ones are fluroquinolones. But the emergency problem of the recent years is the development of fluroquinolone resistance or XDR tuberculosis. The XDR is associated with mutations in the gyrA gene which controles the proliferation of M.tb.
**Aim:** Analysis of mutations of the gyrA gene of M. tuberculosis (M.Tb) strains from patients of penitentiary system and civilian hospitals of Kyrgyz Republic.
**Materials and methods:** Sputum samples of 79 pulmonary tuberculosis (TB) patients were analyzed by biochip assay to detect the gyrA mutations of M.tb. From all samples the 33 sputum samples were taken from patients in the penitentiary system and 46 sputum samples were taken from patients in the civilian sector.
**Results:** M.tb resistant to fluroquinolone were found in 8.7% of TB patients from the civilian sector and in 6% of TB patients from the penitentiary system which gives the resistance to fluroquinolones of about 7.6% in general TB patients subpopulation. The detailed analysis of the mutations of gyrA gene revealed its location mainly in codons 90 and 94. The most frequent mutations was Asp94Gly (66.6%) than it was Ala90Val (16.6%). One case had the combination of both mutations Asp94Gly and Ala90Val.
**Conclusion:** In XDR tuberculosis patients from Kyrgyz Republic the resistance of M.tb to fluroquinolones is mainly caused by Asp94Gly and Ala90Val mutations of gyrA gene.

**P1818**

**Novel sequence-based assay for detection of pyrazinamide-resistant *mycobacterium tuberculosis* in clinical specimens from patients with pulmonary tuberculosis in Russia**

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Pyrazinamide (PZA) is an important first-line antituberculous drug used in all regimens recommended by the WHO. However PZA susceptibility testing is not routinely performed in many laboratories so comprehensive surveillance studies of pyrazinamide resistance are rare. The aim of our study was to determine the occurrence of PZA-resistant *M.tuberculosis*. We developed the diagnostic kit based on direct sequencing of *pncA* gene that was expected to detect all type of mutations. We have examined 143 respiratory specimens obtained from 140 Russian patients with pulmonary tuberculosis. All patients have been classified into 5 groups depending on duration of treatment. The first group consists of patients with newly diagnosed tuberculosis who did not received antituberculous drugs previously or taken them less than a month. The second group included patients from 1 to 3 months of such treatment. PZA-resistance has been found in 9 (10.3%) cases from 87 patients of the first group. The frequency of PZA-resistance of patients from the second group has been amounted to 41.6%. In half of these cases we found heterogeneous populations of bacilli consisted of wild and mutation types. PZA-resistance strains were detected in 7 (36.8%) of 19 patients from the third group with a duration of treatment from 3 to 6 months, in 6 (75.0%) of 8 patients with a duration of treatment from 6 to 9 months, and in 4 (80.0%) of 5 patients receiving antituberculous drugs from 9 to 12 months. These data suggest that the sequencing assay may be useful for the direct and rapid detection of PZA-resistant *M.tuberculosis* in clinical specimens.

**P1819**

**Results of spoligotyping of M. tuberculosis strains isolated in Belarus**

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**Introduction:** Multidrug resistant tuberculosis (MDR-TB) is a severe threat to effective TB-control as well as to successful treatment of the individual patients. The reported incidence of MDR-TB in Belarus is somewhat lower than in its neighboring countries, but the level of MDR-TB causes severe obstacles for the national TB program.
**Objective:** To characterize MDR-TB and pan-susceptible M tuberculosis clinical isolates from TB patients in Belarus by molecular strain typing.
**Methods:** The study totally comprised 163 clinical isolates from patients with pulmonary TB in Belarus, 81 isolates being MDR and 82 being pan-susceptible. The DST at the NRL in Minsk was externally quality assured by the WHO/IUATLD SRL in Stockholm. The molecular characterization of the isolates was carried out in Stockholm by spoligotyping according the protocol described by Kamerbeek et al using a commercial kit (Isogen Bioscience). The octal codes were analyzed using SITVIT2 database.
**Results:** For both the MDR- and the susceptible group, most isolates were shown to belong to the Beijing family, seen in 33/81 (41%) and 26/82 (32%) respectively. In second place, in both groups, came the T1 family, identified in 30/81 (37%) and 16/82 (20%) of the cases. A statistical significant association to MDR-TB was demonstrated for the T1 type with the Fisher's exact test (p-value 0.0151) but not for the Beijing family (p-value 0.2564).
**Conclusions:** The result of this study demonstrated differences in the population structure of strains isolated from patients with MDR- and pan-susceptible TB, respectively. The major cause of MDR-TB is an ongoing transmission of resistant M tuberculosis strains, rather than to an independent development of MDR-TB.

**P1820**

WITHDRAWN

**P1821**

**First experience with novel molecular diagnostic method for detection of *M. tuberculosis* and rifampicin resistance**

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Latvia has middle level notification rate of tuberculosis (TB) and ranks among 27 countries with the highest level of multi-drug resistant TB.
**Aim:** To explore the sensitivity of a newly available molecular diagnostic method GeneXpert® (GX) in early detection of *M.tuberculosis* (MT) and rifampicine (RIF) resistance.
**Methods:** Retrospective case control study. 360 respiratory samples (sputum and/or bronchial washings) from 320 subjects were tested with GX from 13/07/2010 (introduction of the method) till 01/11/2010. New pulmonary TB cases were analyzed.
**Results:** 100 patients had new pulmonary TB. GX was positive in 68 cases, including 25 smear negative [Ziehl-Neelsen (ZN) and/or fluorescence microscopy] cases. Results of culture on liquid media (MGIT®) were available in 15 days, but on solid Levenstein-Jensen (LJ) media in 47.

Table 1. Summary of the MT detection methods

| Method | Tested (N of cases) | Positive (%) |
|---|---|---|
| ZN | 59 | 36 |
| Fluorescence | 100 | 42 |
| GX | 100 | 68 |
| LJ | 100 | 98 |
| MGIT® | 52 | 92 |

2 patients with negative LJ culture were GX positive.
Resistance of MT against RIF on LJ media were detected in 14 cases and matched in 3 cases also with MGIT® method (results available in 80 and 24 days respectively).
In 6 cases out of 14 (40%) when RIF resistance detection concurred with LJ method, results with LJ were available in 68 days.
5 discordant cases (GX RIF resistant, LJ RIF sensitive) currently are extensively analyzed.
**Conclusions:** In addition to conventional smear microscopy methods GeneXpert® improves the diagnostic of pulmonary TB and allows faster detection of RIF resistance.

**P1822**

**A new integrated PCR and microarray lab-on-chip for rapid MDR tuberculosis diagnosis**

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**Introduction** Drug-resistant *M. tuberculosis* (Mtb) strains are a threat to tuberculosis (TB) control worldwide and more advanced, fast and affordable technologies are needed to strengthen laboratory capacity for diagnosis of multidrug resistant (MDR) cases.
**Aims** The aim of the study is to develop a new rapid diagnostic tool for MDR-TB using a lab-on-chip (LoC) platform suitable for testing other poverty related diseases.
**Methods** The LoC (In-Check™) provides an all-in-one device for fast amplification of target DNA followed by hybridization on a low-density microarray.
Mtb and most of the clinically relevant mycobacterial species are identified by specific probes targeting the 16S rRNA gene and IS6110. A multiplex PCR was developed to amplify *rpoB*, *katG*, and *inhA* as the most frequently mutated genes involved in resistance to rifampin and isoniazid in species belonging the Mtb complex.
**Results** The In-Check™ platform was evaluated on isolates and smear positive clinical specimens.
Selected probes allowed identification of Mtb complex, and 10 clinically relevant non-tubercular mycobacterial species, including *M. avium* and *M. intracellulare*. The assay detects the following mutations involved in drug resistance: D516V, S531L (*rpoB*), S315T (*katG*), and c-15t, t-8c, t-8a (*inhA*).
Other mutations at codons 533, 526 (*rpoB*), and 315 (*katG*) are identified by a negative signal from wild-type probes. Detection limit is $10^3$ bacteria/mL.
**Conclusions** The In-Check™ platform represents an innovation for its simplicity of use, rapidity and cost-effectiveness and it is particularly suitable for different diagnostics purposes. This is the first device for molecular detection of malaria and TB on the same platform.

**P1823**

**PCR for diagnosis of tuberculosis and pulmonary mycoses in México**

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**Introduction:** Tuberculosis (TB) and pulmonary mycoses are a public health problem in the north of Mexico. We use polymerase chain reaction (PCR) for diagnosis of TB in patients with a negative baciloscopy. This method also allows us to differentiate between other *Mycobacterium* and microorganisms like the case of *Histoplasma capsulatum, Coccidioides immitis* and *Aspergillus fumigatus.*
**Objective:** The identification of *Mycobacterium tuberculosis* in patients showing clinical and radiological findings with negative baciloscopies and its association with pulmonary mycosis.
**Methods:** Symptomatic patients with high pulmonary TB probability were selected by a complete physical exam, clinical history, thoracic x rays and baciloscopy. In patients with a negative baciloscopy an sputum sample was taken or a Bronchial-alveolar lavage to identify the presence of *Mycobacterium, Histoplasma, Coccidioides* and *Aspergillus* by PCR. Adequate treatment was administered according to the results obtained.
**Results:** A total of 23 patients were analyzed. The presence of *Mycobacterium tuberculosis* was found in 9 cases. In 5 subjects there was of *Aspergillus fumigates* and in 4 cases of both. In 2 cases the presence of *Mycobacterium tuberculosis, Histoplsma capsulatum, Coccidioides immitis* were associated. Three samples were negative for all those microorganisms.
**Conclusions:** PCR's sensibility and specificity increases the diagnosis of TB and mycoses cases. It is a magnificent tool when baciloscopy is negative generating the need for use in the everyday medical practice. The use of innovative techniques for early diagnosis and treatment of frequent pulmonary disease in this region is imperative.

**P1824**

**Relationship between rpoB mutations and minimum inhibitory concentrations of rifampicin in multi drug resistant strains of mycobacterium tuberculosis**

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**Introduction:** Rifampicin (RF) resistance serves as a surrogate marker for detection of multi drug resistant (MDR) strains of Mycobacterium tuberculosis (MTB). Among many mutations identified in rpoB gene, the target gene for detection of RF resistance, few were verified by molecular genetic methods.

**Aim:** To detect and characterize mutations in rpoB region of MDR MTB strains by automated DNA sequencing.
To study the relationship between in vitro Minimum Inhibitory Concentrations (MIC) for RF and rpoB mutations
**Methods:** Absolute concentration method was used to determine MIC of RF for 20 MDR MTB strains.
DNA sequencing was carried out in an ABI sequencer.
**Results:** We could detect point mutations in the 81 bp section of a "hot spot" region of the rpoB gene of all the MDR strains.
Mutations were detected in positions 516,526 and 531, with frequencies of 30%, 40%, and 55%, respectively.
It was found that mutations in positions 526 and 531 conferred high-level resistance to RF (MIC's $\geq$ 128$\mu$g/ml).
Mutations in positions 516 relate to low-level resistance. (MIC's $\leq$ 64$\mu$g/ml).
Double point mutations in 2 isolates in positions 526 & 531 and 1 isolate in position 516 & 531 relate to high level resistance (MIC's $\geq$ 128$\mu$g/ml), while 2 isolates in position 516 & 526 relate to low level resistance (MIC's $\leq$ 64$\mu$g/ml).
**Conclusion:** A relationship between susceptibility to RF and alterations in rpoB gene is observed. However, relationship between gene alteration and drug-resistant phenotype is still unclear, further analysis of the relationship between MICs and gene alteration is necessary.

**P1825**
**Hair-analysis for acetyl-isoniazid/isoniazid ratio and N-acetyl-transferase-2-genotype in patients on treatment for mycobacterium tuberculosis infection**
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**Background:** In the presence of non-compliance, drug malabsorption or widely spaced intermittent therapy genetically determined faster acetylation of isoniazid has been shown to lead to treatment failure and relapse. Hepatotoxicity and peripheral neuritis are associated with slow acetylation of isoniazid. Objectives were to investigate what determines hair-levels of isoniazid and to assess whether acetylator phenotype in form of the isoniazid/acetyl-isoniazid ratio in hair reflects N-acetyltransferase –2 (NAT-2) genotype.
**Patients and methods:** Hair was obtained from patients on isoniazid treatment. Isoniazid and acetyl isoniazid levels in hair were determined using HPLC/MS. Isoniazid/Acetyl isoniazid ratios were correlated with genotype of the NAT-2 determined by PCR. Hair levels of isoniazid were related to age, gender, weight, body mass index (BMI) and ethnic group.
**Results:** Hair levels of isoniazid and acetyl-isoniazid were measured in 40 patients and genotype determined in 24. Hair levels of isoniazid correlated significantly with age and weight ($p<0.05$), but not with BMI or ethnic group. Acetyl-isoniazid/isoniazid ratios were with a median of 15.2% (range 14.5 to 31.7, n=3) in homozygous rapid acetylator NAT-2 genotype and 37.3% (range 1.73 to 51.2, n=7) in the heterozygous rapid acetylator NAT-2 genotype both significantly higher than in the slow acetylator NAT-2 genotype with 5.8% (range 0.53 to 14.4, n=14).
**Conclusions:** Results of hair-analysis for isoniazid showed age and weight dependency of isoniazid levels. Acetyl-isoniazid/isoniazid ratios were lower in patients with slow acetylator versus rapid acetylator genotypes.

**P1826**
**Evaluation of the detection of non-cultivable *mycobacterium tuberculosis* in human clinical samples**
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**Background:** *Mycobacterium tuberculosis* is supposed to stay in a quiescent state when it establishes a latent infection, being unable to grow in culture.
**Objectives:** The aim of this study was to evaluate the detection of noncultivable bacilli in human clinical samples using a procedure that is independent of the immunological status of the patient.
**Materials/Patients and methods:** The study was performed on 61 samples obtained from samples received for routine diagnosis to rule out a mycobacterial infection. Specimens from pulmonary and extra-pulmonary origins were studied by real-time quantitative PCR and the amplification of *Mycobacterium tuberculosis* was observed at the DNA and RNA levels. Clinical records of 52 patients were also reviewed.
**Results:** *Mycobacterium tuberculosis* genomic sequences were detected in all culturepositive samples whereas they were detected in 26% of the culture-negative samples, including 24% with a basal metabolic activity detected.
**Conclusions:** We were able to detect viable but non-cultivable bacilli with a metabolic activity in both pulmonary and extra-pulmonary samples. The amplifications of the *ftsZ* gene and of the main promoter of the ribosomal operon, namely PCL1, seem to be good targets to detect active bacilli putatively involved in latent Tuberculosis.