## 76. Tuberculosis and immunologic assays

**P415**

**Diagnostic performance of the interferon gamma release assay in elderly patients and clinical factors to support the diagnosis of active tuberculosis**

Hiroyuki Kamiya, Soichiro Ikushima, Keisuke Kondo, Kota Satake, Minoru Inomata, Atsuko Moriya, Tsunehiro Ando. *Respiratory Medicine, Japanese Red Cross Medical Center, Tokyo, Japan*

**Background and objective:** The understanding of the diagnostic performance of the interferon gamma release assay in elderly patients is crucial in a country like Japan, where the higher rate of a prior history of tuberculosis can affect the test result.
**Methods:** From a total of four hundred twenty five patients screened, who were suspected of having active tuberculosis and received the QuantiFERON-TB Gold In-Tube test (QFT-GIT), 65 patients in younger age group with the age less than 70 years, and 52 patients in elderly age group were eligible for the analysis. The diagnostic performance of the test was compared between two age groups, and the possible clinical factors to discriminate active tuberculosis cases from elderly patients with positive results of the test were also evaluated.
**Results:** Although the number of patients diagnosed to have active tuberculosis was similar among both age groups, the number of false positive results was significantly higher in elderly patients (4.6% compared to 23.1%). The specificity, positive predictive value and positive likelihood ratio were significantly lower in elderly patients, at 72.7% compared to 93.8%, 36.8% to 83.8%, and 3.21 to 14.12, respectively although other values were almost similar. Radiological findings, such as small nodules and infiltrates, were noted in more cases with active tuberculosis in elderly patients with positive results of the test than with other diseases.
**Conclusions:** The QFT-GIT test may be less accurate in elderly patients in the diagnosis of active tuberculosis, and radiological findings can be helpful in the clinical evaluation of positive results of the test.

**P416**

**New skin test with recombinant protein CFP10-ESAT6 in patients (children and adults) with tuberculosis, non-tuberculosis disease and latent TB infection**

L. Slogotskaya[1], V. Litvinov[1], Ya. Kochetkov[1], E. Ovsyankina[1], D. Kudlay[2], P. Seltsovsky[1], N. Nikolenko[2], D. Ivanova[1]. *[1]Clinical Research, Scientific and Clinical Antituberculosis Center of Moscow Government Health Department, Moscow, Russian Federation; [2]Research, OJSC "Pharmstandard", Moscow, Russian Federation*

DIASKINTEST (DST) – new preparation for skin testing contains recombinant proteins CFP10-ESAT6 being absent in all *M. bovis BCG* substrains and most environmental mycobacteria.
**Aim:** To determine sensitivity & specificity of DIASKINTEST in children & adults with pulmonary, extrapulmonary TB, non-tuberculosis disease, latent TB infection (LTBI) and BCG vaccinated.
**Method:** 2609 patients received Mantoux test with 2 TU PPD-L and DST 0.2 mkg/0.1 ml.
**Results:** 97,3% (178/183) children & adolescents and 84,2% (208/247) adults with pulmonary TB & 89,7% (26/29) with extrapulmonary TB were DST- positive. Among 88 adults with TB/HIV 41 had <200 CD4 + T-lymphocytes (DST-positive were 22,0%) & 47 had >200 count (DST-positive were 55,3%). 94,6% (105/111) with pulmonary and 98,5% (67/68) with extrapulmonary non-tuberculosis disease were DST-negative. All 19 patients with non-active extrapulmonary TB had negative DST reactions.Among 1636 children & adolescents with LTBI the highest percentage of positive DST reactions - 94,9% (37/39) were observed in patients with converted tuberculin reactions and household sputum positive TB contacts, the lowest - 2,2% (16/718) - with close social sputum negative TB contact. In adolescent students with close social sputum positive contact 77,9% were Mantoux - positive & only 5,6% (8/143) were DST- positive - among them 5 TB cases & 3 individuals with LTBI were detected.
DST was not positive in all 228 BCG-vaccinated. Among all children & adolescents 93,8% were Mantoux- positive.
**Conclusion:** DST demonstrated high sensitivity for both active TB and LTBI & high specificity in BCG vaccinated & non-tuberculosis disease.

**P417**

**Prevalence of long-lasting IGRA positivity in Portuguese healthcare workers**

José Torres Costa[1], Chandrasekharan Kesavachandran[2], Albert Nienhaus[2].
*[1]Devision of Allergy Hospital S. João - EPE, Oporto Medical School, Oporto, Portugal; [2]Institute for Healthcare Research in Dermatology and Nursing (IVDP), University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

**Introduction:** Tuberculosis screening in healthcare workers (HCW) is facilitated by the introduction of IGRA. However, uncertainties regarding the interpretation of IGRAs in the serial testing of HCWs remain.
**Method:** At the University of Oporto Medical Centre in Portugal, IGRAs were introduced in HCW screening in 2007. Screening is repeated after unprotected contact with TB patients or annually depending on the risk assessment. The QuantiFERON-TB Gold In-Tube (QFT) is used and the results of the screening are assessed in a standardised database.
**Results:** Between 2007 and 2011, a total of 539 HCWs were tested with IGRA at least three times. 235 HCWs were tested four times. In all three tests, 15.6% were positive, in all four consecutive IGRAs, 10.2% were positive. 49.9% were negative in all three tests and 44.3% were negative in all four consecutive IGRAs. If the concentration of the first IGRA was below 0.2 IU/mL, 72.8% remained negative in the following two IGRAs compared to 26.3% if the first IGRA showed a concentration of between 0.2 and < 0.35 IU/mL. If the first IGRA concentration was 0.7 IU/mL or higher, the following two IGRAs stayed positive in 72.3% of the HCWs compared to 25% of those with a concentration of between 0.35 and < 0.7 IU/mL. Mean time between first and third IGRA was 22 months.
**Conclusion:** The prevalence of persistently positive IGRA is lower than expected. The persistency of positive or negative IGRA results does depend on the concentration observed in the first IGRA. This further underlines the need for a grey zone (e.g. 0.2 – < 0.7 IU/mL) for IGRA interpretation in the serial testing of HCWs.

**P418**

WITHDRAWN

**P419**

**Impact of Quantiferon TB Gold intensity response in active over latent tuberculosis**

Faheem Khan, Orla Cotter, Jim Clair, David Curran, T. O'Connor. *Respiratory Department, Mercy University Hospital, Cork, Ireland Respiratory Department, Mercy University Hospital, Cork, Ireland Microbiology Department, Mercy University Hospital, Cork, Ireland Respiratory Department, Mercy University Hospital, Cork, Ireland Respiratory Department, Mercy University Hospital, Cork, Ireland*

We sought to determine whether the intensity of response in patients with a positive Quantiferon-TB Gold assay (QTF) was predictive of active over latent tuberculosis, and whether other factors determined the intensity of response.

We analyzed positive Quantiferon assays (Cellestis, Carnegie, Australia) performed between July 2009 and April 2011 in the Mercy University Hospital, Cork. The group consisted of 94 patients with latent tuberculosis and 35 patients with active tuberculosis.
There was no difference in the intensity of response between patients with latent and active tuberculosis (p=0.1589). In patients with latent tuberculosis, there were no correlations between age (p=0.353), sex (p=0.476), smoking status (p=0.323), contact history (p=0.612), Mantoux response (p=0.055), Irish nationality (p=0.768), previous BCG vaccination (p=0.504), WCC (p=0.187), peripheral lymphocyte count (p=0.786), neutrophil count (p=0.157) and the intensity of QTF response. Similarly in active TB group there is no correlation found between mentioned variables and QTF response.
The intensity of QTF response does not help to differentiate active from latent tuberculosis. In adults with tuberculosis, the intensity of QTF response is not influenced by age, sex, smoking, remoteness of contact history, Mantoux response, nationality, CXR abnormalities, BCG vaccination and peripheral lymphocyte count.

**P420**

**Tuberculin skin test and IGRA test in the diagnosis and follow-up of bacteriologically confirmed pulmonary tuberculosis in HIV negative adults**

Jiri Homolka, Ivana Hricikova, Emilia Kopecka, Martina Vasakova.
*Pneumological Department, 1st Medical Faculty and Thomayer Hosp., Prague, Czech Republic*

**Introduction:** Tuberculosis (TB) remains a disease of serious concern worldwide. One third of world population is infected with *Mycobacterium tuberculosis complex* and eight to ten million persons develop the disease every year. The very important step to control TB is detecting TB infection by a sensitive test.
**Objectives:** We prospectively evaluated the sensitivity of tuberculin skin test (TST) and IGRA test (Quantiferon-TB Gold) in HIV negative patients with bacteriologically confirmed pulmonary TB. In a subgroup of 40 TB patients we analyzed the results of IGRA test repeatedly (3 months, 6 months, 9 months and 15 months) after completion of AT therapy.
**Results:** Starting January 1, 2008 to December 31, 2011 199 adult patients (mean age 46 yrs ± 18,3 yrs) were included in the study. All patients had positive cultivations for *Mycobacterium tuberculosis*. TST was positive in 125 patients, negative in 65 patients and not done in 9 patients. The sensitivity of TST was 65,8%. IGRA test was positive in 119 patients, negative in 20 patients, indeterminate in 13 patients and not done in 47 patients. The sensitivity of IGRA test was 78,3%. In a subgroup of patients 75% were IGRA positive after 3 months, 65% after 6 months and 40% after 15 months after successful TB treatment.
**Conclusion:** IGRA test showed better sensitivity compared to TST in HIV negative adults with pulmonary TB. The positivity of IGRA test can be observed for a long interval after the completion of AT regimen showing long lasting presence of sensitized T-cells in individuals successfully treated for pulmonary TB.

**P421**

**The responses of multiple cytokines following incubation of whole blood from TB patients, latently infected individuals, and controls with the TB antigens ESAT-6, CFP-10, and TB7.7**

Sae Byol Kim[1], Song Yee Kim[1], Moo Suk Park[1], Yong Sam Kim[1], Se Kyu Kim[1], Joon Chang[1], Hye Jon Lee[2], Sang-Nae Cho[2], Young Ae Kang[1].
*[1]Division of Pulmonology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea; [2]Department of Microbiology, Yonsei University College of Medicine, Seoul, Republic of Korea*

**Purpose:** The development of clinically relevant biomarkers is important for diagnosing latent tuberculosis infection (LTBI) and active tuberculosis (TB) and predicting their prognoses. This study examined whether the responses of multiple cytokines can be used as a biomarker to distinguish the TB infection status and mycobacterial load.
**Methods:** We analyzed the responses of multiple cytokines (IFN-γ, IL-2, IL-10, IL-13, IL-17, and TNF-α) in the supernatant from the QuantiFERON- TB Gold In-Tube assay following stimulation of whole-blood from TB group (n = 32), LTBI group (n = 19), and healthy controls (n = 30) with TB antigens (ESAT-6, CFP-10, and TB7.7).
**Results:** The median responses of IFN-γ, IL-2, IL-10, and IL-13 were higher in the LTBI and active TB groups than in the non-TB control group (IFN-γ, $p < 0.001$; IL-2, $p < 0.001$; IL-10, $p = 0.012$; IL-13, $p < 0.001$). The median IL-2/IFN-γ ratio of the LTBI group was higher than that of the active TB group ($p = 0.014$) and differed significantly among LTBI, smear-negative TB, and smear-positive TB patients ($p = 0.027$). This difference was especially evident between the LTBI and smear-positive TB patients ($p = 0.047$).
**Conclusions:** IFN-γ, IL-2, IL-10, and IL-13 can serve as biomarkers for distinguishing TB infection. In addition, the IL-2/IFN-γ ratio appears to be a biomarker for diagnosing LTBI and may be useful as a prognostic factor and for evaluating treatment responses.

**P422**

**Diagnostic value of gamma interferon assay in tuberculosis pericardial effusions: Study on a cohort of Iranian patients**

Soroush Seifirad[1], Setareh Davoudi[2], Reihaneh Mohsenipour[2], Farzad Masoudkabir[1], Abbas Salehi Omran[3]. *[1]Endocrinology and Metabolic Research Institute, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran; [2]Department of Infectious Diseases, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran; [3]Department of Cardiac Surgery, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran*

**Background:** The efficacy of Interferon-gamma (INF-gamma) and adenosine deaminase (ADA) for diagnosing tuberculosis pericarditis in a cohort of Iranian patients presenting with pericarditis were evaluated.
**Methods:** Thirty eight patients with presentation of pericarditis underwent diagnostic and therapeutic pericardiostomy with drainage and biopsy. Adenosine deaminase and Interferon-gamma levels were determined in pericardial fluid samples of all patients. Pericardial tissue samples were submitted for histopathologic and microbiologic studies. Polymerase chain reaction (PCR) was performed on all pericardial fluid samples to detect Mycobacterium tuberculosis.
**Results:** Among 38 patients with pericarditis, 7 cases were diagnosed as tuberculosis pericarditis (18.4%). Mean concentration of Interferon-gamma in tuberculosis group was significantly higher compared to non-tuberculosis group (69257.14±43427.8 vs. 329.03±433.7, P<0.000). ROC showed a value of 14400 pg/L as cutoff point for INF-γ with a sensitivity of 100% and specificity of 100% for diagnosing tuberculosis pericardial effusion. adenosine deaminase was not found to be significantly higher in tuberculosis group in comparison with non-tuberculosis causes of pericardial effusion (35.7±23.8 vs. 36.03±72.27, P=0.28).
**Conclusions:** According to the results of this study Interferon-gamma showed to be a valuable diagnostic test for detection of tuberculosis pericarditis, while adenosine deaminase measurement did not prove to have the characteristics of an accurate diagnostic test for tuberculosis pericarditis.

**P423**

**Concordance between tuberculin skin testing and interferon-gamma release assays in diagnosis of latent tuberculosis infection among HIV-infected individuals**

Adriana Socaci[1], Gheorghe Nini[2], Voicu Tudorache[1], Teodora Moisil[3], Erdin Borgazi[4]. *[1]Department of Pneumology, Clinical Hospital "Dr.V Babes", Timisoara, Reunion; [2]Department of Pneumology, West University "Dr.V Babes", Arad, Romania; [3]Department of Infectious Diseases, Clinical Hospital "Dr.V Babes", Timisoara, Romania; [4]Department of Pneumology, Clinical Hospital Pneumophtysiology, Constanta, Romania*

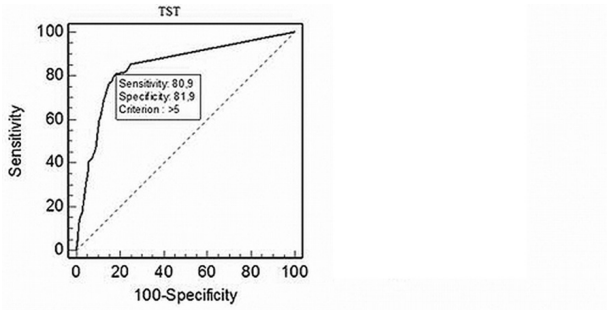
**Introduction:** Detection and treatment of latent TB infection (LTBI) in HIV infected individuals is strongly recommended to decrease morbidity and mortality in countries with high levels of HIV. Interferon gamma release assays (IGRA) are now available alternatives to tuberculin skin test (TST) to detect LTBI.
**Aims:** This study compared QuantiFERON-TB Gold In-Tube (QFT-IT) with the TST for the detection of latent tuberculosis infection among HIV-infected individuals in an area with a high prevalence of tuberculosis (120%000), where BCG vaccination is mandatory.
**Methods:** A prospective study of HIV-infected individuals with received the TST and an IGRA, the QFT-IT.
**Results:** Of 147 participants, 106 (72.1%) returned for an evaluable TST. Concordance between QFT and TST was 88.8% (kappa = 0.37, p ≤0.001). However, in subjects with positive test results by either TST or QFT, only 27% (4/15) had positive test results by both modalities. TST-positive/QFT-negative discordant results were found in 5.1% of subjects and TST-negative/QFT-positive discordance in 6.1%. Indeterminate QFT results occurred in 5.4% (8/147), all due to a failure to respond to the phytohemagglutinin-positive control. Subjects with a CD4+ count of less than 100 cells/mm$^3$ had a relative risk of an indeterminate result of 4.24 (p= 0.003) compared with those with a CD4+ count of 100 or more.
**Conclusions:** Overall concordance between QFT and TST in HIV infection was high and similar to that seen in an immunocompetent population, but QFT testing may be limited by an elevated rate of indeterminate results in subjects with CD4+ cell counts of less than 100 cells/mm$^3$.

**P424**

**Cut-off point for tuberculin skin test (TST) and QuantiFERON (QTF) in the diagnosis of tuberculosis infection (TI) in a study of contacts of tuberculosis (TB)**

Jose-Maria Garcia-Garcia[1], Juan-Jose Palacios[2], Begoña Gonzalez-Gonzalez[3], Ana Fernandez-Quiroga[3], Henar Villar[4], Manuel Villanueva[1], Gema Castaño[1], Kaoutar El Boutaibi Faiz[1], Fernando Alvarez-Navascues[1], Andrés Sanchez-Antuña[1], Jose-Antonio Gullon[1], Jesus Allende[1], Manuel Martinez-Muñiz[1], Jose-Manuel Fernandez-Carreira[5]. *[1]Area Gestión Clinica Neumologia, Hospital San Agustín, Aviles, Asturias, Spain; [2]Unidad Referencia Micobacterias, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain; [3]Atención Primaria, Area Sanitaria III, Aviles, Asturias, Spain; [4]Microbiología, Hospital San Agustín, Aviles, Asturias, Spain; [5]Unidad Investigacion, Hospital San Agustin, Aviles, Asturias, Spain*

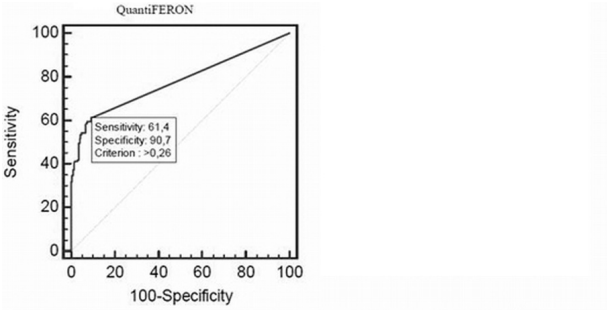
**Aim:** To determinate the cut-off for TST assuming a diagnosis of TI when QTF was ≥ 0.35 IU/mL Moreover, we calculated the cut-off for QTF assuming the diagnosis of TI when TST was ≥ 5 mm.
**Methods:** We studied prospectively 414 close contacts from 82 TB patients. We performed "QuantiFERON TB GOLD in Tube " (according to the manufacturer's instructions, Cellestis, Australia) and the same day after venous blood puncture for QTF, TST (Mantoux technique with 2 UI of PPD RT23 with lecture 72 hours later). We studied sensibility, specificity and analysis "Receiver Operador Characteristics" (ROC). The positive and negative predictive values (PPV, NPV) were calculated based in our proportion of TI of 52,9%.
**Results:** Assuming as "gold standard" of TI a value of QTF 0.35, the best cut-off of TST was 5mm.




| Continuos variable | TST | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Classification variable | Quantiferon >= 0,35 | | | | | | | |
| Criterion value | Sensibility | 95% CI | specificity | 95% CI | PPV | 95% CI | NPV | 95% CI |
| >5 * | 80,87 | 72,5 - 87,6 | 81,89 | 76,7 - 86,3 | 83,4 | 77,4 - 88,3 | 79,2 | 72,6 - 84,8 |
| >10 | 69,57 | 60,3 - 77,8 | 87,17 | 82,5 - 90,9 | 85,9 | 79,6 - 90,9 | 71,8 | 65,3 - 77,7 |
| >15 | 46,96 | 37,6 - 56,5 | 90,94 | 86,8 - 94,1 | 85,3 | 77,3 - 91,4 | 60,4 | 54,3 - 66,3 |

Figure 1. ROC curve, TI gold stantdard QTF ≥0.35"

Assuming as "gold standard" of TI a value of 5mm, the best cut-off of QTF was 0.26.




| Variable | QUANTIFERON | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Classification variable | TST >=5 mm | | | | | | | |
| Criterion value | Sensibility | 95% CI | Specificity | 95% CI | PPV | 95% CI | NPV | 95% CI |
| >0,26 * | 61,44 | 53,2 - 69,2 | 90,75 | 86,2 - 94,2 | 88,2 | 81,6 - 93,0 | 67,7 | 61,4 - 73,6 |
| >0,35 | 59,48 | 51,3 - 67,3 | 90,75 | 86,2 - 94,2 | 87,8 | 81,1 - 92,8 | 66,6 | 60,3 - 72,5 |

Figura 2. ROC curve. TI "gold standard" TST 5 mm.

**Conclusion:** 1. In a study of TB contacts the cut-off for TST of 5 mm was the best for the diagnosis of TI.
2. Although the established cut-off for QTF is 0.35, lower values as 0.26 can be taken into account.

**P425**

**Evaluation of cut-off values of QuantiFERON-TB Gold, interferon gamma inducible protein-10 and tuberculin skin test in active tuberculosis diagnosis**

Ekrem Seyhan[1], Kaya Köksalan[2], Gülsah Günlüoglu[1], Sinem Sökücü[1], Nurdan Veske[1], Aysegül Akbas[1], Sedat Altin[1]. *[1]Chest, Yedikule Chest Diseases and Thoracic Surgery Education and Research Hospital, Istanbul, Turkey; [2]Epidemiology, Molecular Tuberculosis Epidemiology Laboratory, Institute for Medical Experimental Research (DETAE), Istanbul University, Istanbul, Turkey*

**Aim:** The diagnostic accuracy of interferongamma- based assays for Mycobacterium tuberculosis infection may be improved by using lower cut-off values for the tuberculin skin testing (TST), QuantiFERON-TB Gold (QFT) and Interferon gamma inducible protein (IP)-10.
**Methods:** A total of 70 adult TB patients and 81 healthy controls were included for this study.Three assays, TST, QFT and (IP)-10, were evaluated for their diagnostic performance with respect to different cut-off values. Test cut-offs were established based on receiver operating characteristic curve analysis.
**Results:** The sensitivities of the assays were: TST 40%, QFT 87% and IP-10 85%, while their specificities were TST 58%, QFT 71% and IP-10 74%. Both QFT

and IP-10 were significantly more specific than TST (both P< 0.001), but were similiar to each other (P > 0.5). Receiver operating characteristic analysis revealed that a cut-off value of 0.29 IU/ml for QFT and 1857 IU/ml for IP-10 maximises specificity without significant loss of test sensitivity. Using lower cut-off values for TST, however, also increased the sensitivity of the assay but resulted in a significant decrease in specificity.
**Conclusions:** Lower cut-off values for TST, QFT and (IP)-10 increased the sensitivity of each assay, but a lower cut-off value for QFT and (IP)-10 could specificity be maintained.

**P426**
**Sensitivity of the QuantiFERON-TB Gold test in culture-verified NTM disease and TB in a Danish setting**
Thomas Stig Hermansen[1], Vibeke Østergaard Thomsen[2], Pernille Ravn[3]. *[1]Epidemiklinikken, Dep. of inf. Diseases, Rigshospitalet, Blegdamsvej 9, Copenhagen, Denmark; [2]International Reference Laboratory of Mycobacteriology, Statens Serum Institut, Copenhagen, Denmark; [3]Clinical Research Center, Hvidovre Hospital, Kettegard Alle 30, Hvidovre, Denmark*

**Introduction:** Previous studies have suggested that the QuantiFERON®-TB Gold (QTF) test can be used to discriminate between tuberculosis (TB) and non-tuberculous mycobacterial (NTM) disease. The QFT-test has a higher specificity and sensitivity for infection with mycobacterium tuberculosis (MTB) compared to the tuberculin skin test, but only few studies have included a large number of patients on a nation-wide basis.
**Methods:** We have studied 12000 QTF-tests obtained between 2009-2011 from a Danish national database. Results of mycobacterial cultures were avaliable for 300 patients. Perfomance of the QTF-test in the group of patients with active TB and NTM disease was evaluated.
**Results:** 202 patients had positive culture for M. tuberculosis complex (MTC) and a QFT done. We found 165 positive, 29 negative and 8 indeterminate results, resulting in a sensitivity of 81,7%. In total 98 patients had culture verified NTM infection (species known to share the ESAT6 and CFP10 antigens were excluded, n=6). We found 15 positive, 68 negative and 9 indeterminate results. The causative microorganisms of NTM pulmonary disease were: *M. avium* (52%), *M.gordonae (17%), M. Cellatum (7%),M. Malmoense (6%), M. xenopi* (4%), *M. intracellulare* (4%), *M. kansasii (4%).*
**Conclusion:** The sensitivity of the QFT-test in Denmark, a low-burden TB setting; corresponds well with ealier findings. In a large population of patients with NTM we found a specificity of 74% for infection and a relatively high indeterminate rate. The impact of prior BCG vaccination, MTB exposure and immunodeficiency on specificity and indeterminate rate in the NTM group will be further explored.

**P427**
**Treatment in inflammatory bowel disease affects IGRA performance**
Miguel Arias[1], Sabino Riestra[2], Ruth DeFrancisco[2], Ana Pando[1], Juan José Palacios[3], Patricio Escalante[4], José Belda[6], Luis Molinos[1], Marta García-Clemente[1], Begoña Palomo[1], Luis Rodrigo[2], Amador Prieto[5], Ana Losada[1], Pere Casan[1]. *[1]Pulmonary Service, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain; [2]Inflammatory Bowel Disease Unit, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain; [3]Microbiology Service., Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain; [4]Pulmonary and Critical Care Service, Mayo Clinic, Rochester, MN, Spain; [5]Radiology Service, Hospital Universitario Central de Asturias, Oviedo, Asturias, Spain; [6]Pulmonary Service, Hospital Arnau de Villanova, Valencia, Spain*

**Introduction:** The detection LTBI in patients with inflammatory bowel disease (IBD) before treatment with anti-tumor necrosis factor α must be made with chest radiograph and TST. In immunocompromised patients the limitations of this strategy are well known, therefore it is advisable to use new diagnostic methods based on the release of interferon-γ (IGRA).
**Material and methods:** 204 IBD patients underwent screening for detection of LTBI where T-SPOT.TB (T.SPOT) and QuantiFERON-TB Gold In Tube (QTF) determinations were performed simultaneously, also lymphogram, TST and chest X-ray were performed. ITL was defined when the TST and/or any IGRA was positive.
**Results:** 156 patients had Crohn disease, 42 ulcerative colitis and 6 non-specific colitis. 34 were treated with 5-aminosalicylic acid, 63 with immunomodulators, 32 anti-TNF-α, 27 corticosteroids and 47 a combination of them. 14 were positive QFT, 24 for the T-SPOT and 55 for the TST. The lymphogram showed an association between TSPOT + and QTF + and the amount of circulating CD8 (> 500 cells) while QFT- SPOT + results have the amount of CD8 reduced (<500). Moreover, their treatment modified all lymphocyte populations particularly the IMS and corticosteroids.
**Conclusion:** The immunomodulatory treatment of patients with IBD altered lymphocyte profile which, in turn, is related to the result of testing LTBI. A correct interpretation of the results for the study of LTBI needs to know the treatment received and requires an assessment of lymphocyte populations that verifies its normality. If the lymphocytes are low, particularly CD8, the effectiveness of IGRA (specially the QTF), is very small and require every test possible to rule out LTBI.

**P428**
**Application of intracellular cytokine flow cytometry in the diagnosis of active tuberculosis**
Jaehee Lee[1], Eun Jin Kim[2], Seung Soo Yoo[1], Shin Yup Lee[1], Seung Ick Cha[1], Jae Yong Park[1], Chang Ho Kim[1]. *[1]Internal Medicine, Kyungpook National University, School of Medicine, Daegu, Korea; [2]Internal Medicine, Cha University Hospital, Gumi, Korea*

**Background:** Intracellular cytokine flow cytometry (ICCFC) has been introduced to detect the T cell response to *M. tuberculosis* antigen (MTB Ag) to overcome the limits shown by whole-blood interferon-gamma (IFN-γ) release assays (IGRA). Given the important role of CD4+T cells as well as IFN-γ and TNF-α in pathogenesis of TB, we compared the diagnostic accuracy between ICCFC measuring frequencies of MTB-specific Ag stimulated IFN-γ+TNF-α+CD4+T cells and IGRA to confirm the usefulness of application of ICCFC for TB diagnosis in clinical practice.
**Methods:** Both QuantiFERON® TB Gold In-Tube (QFT-IT) test and ICCFC analysis were performed in 80 patients who were suspected of having pulmonary TB or TB pleurisy and 10 controls with no known exposure to TB.
**Results:** (1) Sixty one and 19 out of total 80 patients were diagnosed with active TB and non-TB, respectively. (2) Double IFN-γ+TNF-α+CD4+T cells among all T cell subsets analyzed by ICCFC showed the highest sensitivity (90%) for diagnosis of TB. (3) Sensitivity of QFT-IT test and ICCFC assay were 77% and 90%, respectively (p = 0.021). (4) Specificity of QFT-IT and ICCFC assay were 73.7% and 89.5%, respectively. (5) There was a good correlation between the quantitiy of IFN-γ, as detected by the QFT-IT test, and the freqeunceis of IFN-γ+TNF-α+CD4+T cell measured by the ICCFC assay in TB patients (p=0.012). (6) The frequencies of IFN-γ+TNF-α+CD4+T cells were significantly decreased after 6 months of treatment compared with pretreatment (p=0.026).
**Conclusions:** The ICCFC assay with T cells stimulation by MTB-specific Ag may be a useful additional tool for the diagnosis of active TB, although further study is needed for more convincing data.

**P429**
**Immune responses in the lungs of patients with tuberculous pleural effusion without parenchymal tuberculosis**
Won-Il Choi. *Internal Medicine, Keimyung University Dongsan Medical Center, Daegu, Republic of Korea*

**Background:** Tuberculous pleural effusion (TPE) is one of the most common forms of extrapulmonary tuberculosis. Because most studies of TPE focused on the pleural space, little information regarding lung parenchyma is available. We therefore aimed to investigate immune responses in the lung parenchyma of TPE patients without active parenchymal tuberculosis.
**Methods:** Patients with any evidence of active parenchymal tuberculosis, either from radiologic or bacteriologic evaluation, were excluded. Bronchoalveolar lavage fluid (BALF) was collected from 10 newly diagnosed, untreated, HIV-negative TPE patients and 10 healthy controls. We analyzed T-lymphocyte subpopulations and measured 10 cytokines in BALF. Cytokine levels in BALF were standardised using urea.
**Results:** The concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), and the CD4+/CD8+ ratio of T-lymphocytes were significantly higher in TPE patients without active parenchymal tuberculosis than in the controls. Of the cytokines measured in BALF, VEGF showed the highest concentration. No difference was observed in T-helper type 2 cytokines between the 2 groups.
**Conclusion:** There were significant immune responses and increases in IFN-γ, TNF-α, and VEGF in the lung parenchyma of TPE patients without active parenchymal tuberculosis. This result suggests that TPE may induce a significant immune response in lung parenchyma.

**P430**
**Comparison of interferon gamma assay and tuberculin skin test in household TB contacts**
Svetlana Kasikovic Lecic, Slobodan Pavlovic, Vesna Kuruc, Miroslav Ilic, Darinka Kukavica. *Clinic for Granulomatous Diseases, Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Voivodina, Serbia*

**Background:** Identification and treatment of active and latent TB infection among household contacts is an effective strategy for TB control.
**Aim:** To compare the QuantiFERON-TB Gold in tube assay (QFT) to tuberculin skin testing (TST) for detecting TB infection among household contacts.
**Methods:** The participants were 50 immunocompetent household TB contacts. All the participants were interviewed using a questionnaire and tested by the QFT and TST. TST results were analyzed at 5 mm and 15 mm cut-off. The QFT test was interpreted following the manufacturer's criteria. In all the subjects with a positive TST (≥15mm)/QFT, X-ray was performed.
**Results:** All contacts had previously been vaccinated and revaccinated with BCG and they had been screened with the TST at least once in the past. 52% (26/50) household contacts had positive TST (≥5mm) results, and 12% (6/50) had TST≥15mm. 18% (9/50) had a positive QFT finding. The comparison of TST (threshold 5 mm) and QFT results revealed a low agreement: 62% (31/50); (k=0.259). The comparison of TST (threshold 15 mm) and QFT results revealed

a moderate agreement: 90% (45/50); (k=0.611). Active TB was detected in 4% (2/50) subjects.
**Conclusion:** Despite the intense exposition of immunocompetent household TB contacts to a highly symptomatic index case, transmission of M. tuberculosis rarely occurred. The data indicate that a previous BCG vaccination and/or TST testing could be a reason for the false positive TST results. Two tests can be used in the diagnostic algorithm of TB infection in testing household contacts. In order to apply the TST a higher cut off values (≥15 mm) should be used for positivity. The QFT assay could replace the TST in BCG vaccinated population.

**P431**
**Experience of using Diaskintest by tuberculosis patients**
Liudmila Pankratova, Natalia Kazimirova, Ksenia Klochkova, Margarita Ogareva.
*Chair of Phthisiopulmonology, Saratov State Medical University, Saratov, Russian Federation*

This is a study of Diaskintest (DST) - a new diagnostic test for tuberculosis (TB). It is an additional method of TB diagnosis and of evaluation of activity of the TB process. The objective of the study was to evaluate the sensitivity and specificity of the test on a sample of TB patients treated at the TB hospital in Saratov (Russia) in 2010 - 2012. A total of 95 subjects participated: newly diagnosed - 69.5%, with relapses - 11, 6%, chronic forms -18.9%. HIV co-infection in 22.1%.
In most TB patients (71.6%) DST was found to be positive. Test average value was 9,8±8,85, including M 9,0±8,21 in newly diagnosed subjects, 11,5±11,71 in subjects with relapses, and 11,6±9,41 in chronic patients. In subjects with limited processes, DST was 7,44±4,91, and in subjects with wide-spread and destructive processes - 10,6±9,77 ($p \geq 0,05$). Only 42.9% subjects with HIV infection, were DST-positive. Out of the 95 participants, 27 subjects with clinically confirmed TB, 28.4% were DST-negative. Of these, 12 had HIV infection which could have caused suppression of DST sensitivity. Of the remaining 15 subjects with TB, 10 were confirmed by bacterioexretion, one – by histological examination of surgical specimens, and only 4 subjects did not have such confirmation. In other words, in 11 cases (11.6%) the presence of tuberculosis was not revealed by DST.
**Conclusion:** DST was positive in 71.6% of TB cases, but only 42.9% among HIV-positive subjects, and in 79.7% in subjects without HIV infection. In 11.6% of the cases, DST did not reveal the presence of TB.