197. New methods to diagnose tuberculosis infection

2173

Gamma-interferon tests for the diagnosis of tuberculosis infection under routine conditions in Lausanne, Switzerland

Ariane Zellweger[1], Sandrine Ansermet[1], Stephanie Jolivet[1], Jean-Pierre Zellweger[2]. *[1]Dept of Immunology, BBR Analytic Laboratories, Lausanne, Switzerland; [2]Pneumology and Tuberculosis, University Medical Policlinic, Lausanne, Switzerland*

Aim: To describe the experience with the performance of Gamma-Interferon tests for the detection of tuberculosis infection under routine clinical conditions in a private analytical laboratory in Lausanne, Switzerland

Method: Retrospective analysis of all T-SPOT.TB tests performed in 2005, on request of the local Office of Public Health and Lung Association in charge of contact tracing for tuberculosis, local hospitals, private physicians and organizations caring for immigrants.

Results: We performed 1435 tests, of which 396 (28%) were positive, 880 (68%) negative and 59 (4%) indeterminate. The main indications were contact tracing among contacts of tuberculosis patients, screening of immigrants with positive tuberculin skin tests, surveillance of health care workers, search for latent infection among immunosuppressed patients and TB suspects. By age groups, tests were more frequently indeterminate among children < 5 years (5%) and adults > 60 years (11%). Most indeterminate tests were due to the lack of stimulable lymphocytes.

Proportion of positive, negative and indeterminate T-SPOT.TB tests, by age group

age group (years)	0-5	6-14	15-29	30-59	60 +	Total
positive	3 (16%)	9 (29%)	100 (35%)	215 (15%)	69 (30%)	396 (28%)
negative	15	22	181	630	132	880 (68%)
indeterminate	1 (5%)	0	5 (2%)	28 (3%)	24 (11%)	59 (4%)
total	19	31	286	873	226	1435

Conclusions: Gamma-Interferon tests can be performed under routine clinical conditions. The proportion of indeterminate results is low, possibly increasing with age and associated comorbidity or lack of stimulable lymphocytes. Above the age of 6 years, positivity is unrelated to age.

2174

Validation of the tuberculin skin test cut off in BCG-vaccinated individuals using the QuantiFERON-TB gold test

Roberto Piro[1], Giovanni Ferrara[1], Alessandro Andreani[1], Marisa Meacci[2], Barbara Meccugni[2], Ilaria Marchetti[2], Monica Losi[1], Leonardo M. Fabbri[1], Luca Richeldi[1]. *[1]Dep. of Oncology, Haematology and Pneumology - University of Modena and Reggio Emilia, Section of Respiratory Disease, Modena, MO, Italy; [2]Policlinico Hospital, Laboratory of Microbiology and Virology, Modena, MO, Italy*

Background: BCG vaccination is widely used around the world and it is known to cause falsely positive tuberculin skin test (TST) reactions. Current cut off values of the TST among BCG-vaccinated subjects are based on retrospective evaluations of large screening studies, but they have not been validate yet using a more specific assay. We have therefore evaluated the results of the TST and of

the *M. tuberculosis*-specific QuantiFERON-TB Gold (QFT-G) test in a group of BCG-vaccinated individuals.
Methods: The results of both TST and QFT-G in all patients tested in the period between November 2003 and August 2005 were evaluated. The distribution of the positive QFT-G tests was investigated stratifying the results for the diameter of the TST, using different cut off values.
Results: Seven-hundred-eleven patients were tested with both tests: of them, 131 (18.4%) were BCG-vaccinated. Among the BCG-vaccinated individuals, the rate of positive QFT-G was significantly higher in those with a a TST≥10 mm (39,7% *vs* 11.3% in those with a TST<10 mm, OR 5.2, p<0.001); however, no difference was found using a 5 mm cut off value for the TST (p=0.10). On the other hand, in subjects with a TST result >5 and <10 mm, the rate of positive QFT-G tests was significantly higher among non-vaccinated individuals, compared to BCG-vaccinated subjects (51.1% vs 12.8% respectively; p<0.001).
Conclusions: The results of the *M. tuberculosis*-specific QFT-G test lend support to the use of the 10 mm value as a valid cut off for the diagnosis of latent tuberculosis infection in BCG-vaccinated individuals.

2175
Latent tuberculosis infection among respiratory and clinical staff and students in Samara, Russia
Yanina Balabanova[1], Ivan Fedorin[2], Vladislav Nikolayevskyy[1], Svetlana Zakharova[3], Natalya Lebedeva[4], Elena Zakamova[2], Francis Drobniewski[1]. *[1]Mycobacterium Reference Unit, Institute of Cell and Molecular Science, Barts and Queen Mary Shcool of Medicine, London, United Kingdom; [2]Samara Regional Tuberculosis Service, Samara Regional Tuberculosis Dispensary, Samara, Russia; [3]Samara Regional Tuberculosis Service, Samara City Tuberculosis Dispensary N1, Samara, Russia; [4]Samara Regional Tuberculosis Service, Samara City Tuberculosis Dispensary N2, Samara, Russia*

Background: Samara is one of 89 regions of Russia with a general tuberculosis (TB) incidence rate of 69.3/100 000 and high rates of drug resistance but the incidence among TB workers is ten times higher (741.6/100 000).
Aim: to measure the prevalence of latent TB among medical staff and students using novel ex-vivo cellular gamma interferon assay.
Methods: a cross-sectional study amongst clinical staff and students using the Quantiferon Gold In-tube assay system (Cellestis, Australia).
Results: the rates of LTBI are shown in Table 1. Positivity was significantly higher in health care workers compared to the control group (OR-15.2; 95%CI 9.8-23.6). TB doctors and nurses had significantly higher rates compared to primary health care staff (OR-2.1; 95%CI 1.2-3.8). Age, work at TB facilities and number of years of TB work were significantly associated with having LTBI.

Rates of LTBI among health care staff and students

Group	N positive, % (95%CI)
All students (n-368)	32 (8.7%; 6.1-11.9%)
Non-medical students (n-130)	8 (6.2%; 2.9-11.3%)
Medical students (n-238)	24 (10.1%; 6.7-14.4%)
All health care staff (n-262)	155 (59.2%; 53.1-65.0%)
Primary care staff (n-122)	38 (31.1%; 23.4-39.8%)
TB staff (n-140)	69 (49.3%; 41.1-57.5%)

Conclusion: the rates of latent infection are extremely high among health care workers in Samara compared to students. The QuantiFERON-TB Gold assay could have a useful role in regular screening of professional groups. Studies are needed to evaluate efficiency of chemoprophylaxis based on gamma-interferon assays results.

2176
Contribution of the T-SPOT.TB assay for differentiating active versus latent tuberculosis infection (LTBI)
Valerie Bosshard[1], Pascale Roux-Lombard[2], Thomas Perneger[3], Marie Metzger[1], Regis Vivien[2], Thierry Rochat[1], Jean-Paul Janssens[1]. *[1]Pulmonary Diseases, Geneva University Hospital, Geneva, Switzerland; [2]Allergology and Immunology, Geneva University Hospital, Geneva, Switzerland; [3]Social and Preventive Medicine, Geneva University Hospital, Geneva, Switzerland*

Introduction: IFN-gamma tests (T-SPOT.TB) are highly specific for M. tuberculosis complex. It is not known however if these blood assays can distinguish between active TB and LTBI.
Setting: Prospective study of subjects screened during contact-tracing procedures and cases of active TB between 10.04 and 1.06.
Methods: Comparison of quantitative results of T-SPOT.TB assays in patients with active TB (culture +) vs. contacts (Mann Whitney test and ROC curve analysis). T-SPOT.TB assay was performed during the first 2 weeks of treatment for active TB cases. T-SPOT.TB positivity was defined as a > 6 spots difference between Panels containing antigens ESAT-6 or CFP-10 and negative control.
Patients: 59 HIV-negative patients with culture positive active TB; 309 contacts screened for LTBI (aged 40±13 years, 52% M, 87% BCG-positive).
Results: see table.
Active TB vs. contacts with T-SPOT.TB +(TST + or -): p=0.006 (Mann-Whitney). AUC for ROC curve: 0.69 (95%CI: 0.60-0.77). Sensitivity (Se) and specificity (Sp) at various cut-off levels: 20: Se: 0.80, Sp: 0.54; 40: Se: 0.54, Sp: 0.70; 65: Se: 0.51; Sp: 0.79

Number of spots by group	Mean	SD	Number of cases
Active TB	70.4	58.3	59
Contact, TST+, T-SPOT.TB +	49.9	57.1	91
Contact, TST -, T-SPOT.TB +	20.1	25.9	32
Contact, TST +, T-SPOT.TB -	0.9	25.9	75
Contact, TST -, T-SPOT.TB -	1.0	1.7	111

Conclusion: Although number of spots (i.e.: production of IFN-gamma by peripheral lymphocytes exposed to specific antigens) was significantly higher in HIV-negative patients with active TB than in LTBI, there was a considerable overlap in results and thus sensitivity and specificity of T-SPOT.TB in diagnosing active vs. latent TB were moderate.

2177
Do results of T-SPOT.TB assays change during and after treatment of active tuberculosis?
Jean-Paul Janssens[1], Pascale Roux-Lombard[2], Thomas Perneger[3], Marie Metzger[1], Regis Vivien[2], Thierry Rochat[1]. *[1]Pulmonary Diseases, Geneva University Hospital, Geneva, Switzerland; [2]Allergology and Immunology, Geneva University Hospital, Geneva, Switzerland; [3]Social and Preventive Medicine, Geneva University Hospital, Geneva, Switzerland*

Introduction: It has been suggested that blood assays quantifying production of IFN-gamma by lymphocytes exposed to antigens specific of M. tuberculosis (T-SPOT.TB) may be useful for monitoring efficacy of treatment in active TB (1).
Setting: Prospective study of all cases of active TB between 10.04 and 1.06.
Methods: Comparison of quantitative results of T-SPOT.TB in patients with active TB (culture +) during the first 2 weeks of treatment (T0), at end of treatment (TE) and 6 months after end of treatment (TE+6)(Mann Whitney test). T-SPOT.TB positivity was defined as > 6 spots difference between Panel A or B (lymphocytes exposed to ESAT-6 or CFP-10) and negative control.
Patients: 81 HIV-negative patients aged 36 ± 16 years, 46% M, all with culture proven active tuberculosis, and favourable clinical outcome.
Results: Samples were available at T0 for 54 patients, at TE for 43 patients, and at TE+6 for 23 patients. Mean results of T-SPOT.TB for the 3 groups were (Mean ± SD): 75 ± 58, 46 ± 55 and 33 ± 46 spots. Positivity rate of T-SPOT.TB for the 3 groups was 96%, 88% and 91%. Wilcoxon's paired test showed a significant decrease of spots between T0 and TE (p<.001) and a non-significant decrease between TE and TE+6 (p=.09).
Conclusion: These preliminary results do not confirm the hypothesis that T-SPOT.TB results can become negative under adequate treatment for active TB. The decrease in number of spots between T0 and ET suggests that the test correlates with disease activity. However, the clinical utility of this observation is unclear.
(1): Lalvani A. Counting antigen-specific T cells: a new approach for monitoring response to tuberculosis treatment? Clin Infect Dis 2004; 38:757–759.

2178
Monitoring of ESAT-6/CFP-10 selected peptides response during prophylaxis in individuals exposed to *M. tuberculosis*
Delia Goletti[1,6], Donatella Vincenti[1], Stefania Carrara[1], Ornella Butera[1], Federica Bizzoni[1], Massimo Amicosante[4], Duilio Dainotto[3], Nicola Petrosillo[6], Mario Pasquale Parracino[2], Gianfranco Anzidei[5], Enrico Girardi[2]. *[1]Translational Research Unit, National Institute for Infectious Diseases "L. Spallanzani", Rome, Italy; [2]Epidemiology Department, National Institute for Infectious Diseases "L. Spallanzani", Rome, Italy; [3]Pneumology Department, ASL RM3, Rome, Italy; [4]Department of Internal Medicine, University of Tor Vergata, Rome, Italy; [5]Pediatric Division of Health Department, INMI, Rome, Italy; [6]Second Division of Health Department, INMI, Rome, Italy*

Background: We set up a new potential immune assay for diagnosing tuberculosis (TB) and monitoring therapy, able to discriminate between active TB and latent infection. This test measures the IFN-gamma production in response to peptides selected from ESAT-6 and CFP-10 (RD1) proteins. The objectives of the present study were: i) to evaluate the response to this assay in contacts of active pulmonary TB patients; ii) to compare our test with QuantiFERON TB-Gold assay (QFT-G); iii) to monitor these responses during anti-TB prophylactic therapy.
Methods: A total of 193 contacts were tested by RD1 selected peptides Whole Blood ELISA (WBE) and QFT-G at the time of TB diagnosis of the index case.
Results: Among all contacts, 114 were tuberculin skin test (TST) positive and 32 of them started prophylaxis (INH) which is still ongoing in 17. Among the 32 TST+ that started therapy, 20 (63%) responded to RD1 selected peptides and 24 (75%) to QTB-G. Thirteen subjects under therapy, with a positive response to both assays, were studied after 1 and 6 months of treatment (the study is still ongoing). A dramatic decrease (63%) of RD1 selected peptides response was observed after one month of prophylaxis, vs a lower reduction (16%) observed with QTB-G. After completion of therapy a significant decline (>70%) by both assays was found.
Conclusions: Our assay based on RD1 selected peptides may offer an accurate approach for monitoring *M. tuberculosis* replication after exposure. This assay

may be a potential tool for an earlier evaluation of the effect of prophylaxis in contacts of TB patients.

2179

Relationship between the whole blood interferon-gamma responses and the risk of active tuberculosis

Kazue Higuchi, Nobuyuki Harada, Toru Mori. *Immunology Division, Research Institute of Tuberculosis, Kiyose, Tokyo, Japan; Immunology Division, Research Institute of Tuberculosis, Kiyose, Tokyo, Japan; President, Research Institute of Tuberculosis, Kiyose, Tokyo, Japan*

Objective: The new diagnostic method for *M. tuberculosis* (MTB) infection, QuantiFERON®-TB Gold (QFT), measures interferon-gamma (IFN-γ) produced in whole blood stimulated with the MTB specific antigens ESAT-6 and CFP-10. Although IFN-γ responses above the test cut off are diagnostic for MTB infection, the relationship between level of IFN-γ response and the risk of progression to active tuberculosis (TB) is unknown. We analyzed this relationship among QFT positive individuals identified in a contact investigation.
Subjects and methods: People exposed to an index case with active pulmonary TB were tested using QFT. The IFN-γ responses of those who developed TB were compared with those who did not develop TB.
Results: Among 135 subjects, 90 (67%) were QFT positive. All subjects were evaluated for active TB by chest X-ray examination at the time of QFT testing and 19 were diagnosed with active TB based on radiographic abnormalities consistent with TB. All of these 19 subjects were QFT positive, and their average level of IFN-γ production was 6.53±7.26 IU/ml compared with 4.21±4.81 IU/ml for those who did not develop TB; not significantly different. However, when all QFT positive responders were classified into two groups (low responders and high responders) based on an arbitrary IFN-γ cut off of 5 IU/ml, the rate of TB development in high responders (33.3%) was twice that seen in low responders (15.9%: p=0.063).
Conclusion: Our results suggest that subjects with high levels of IFN-γ production in response to either ESAT-6 and/or CFP-10 in the QFT test have a higher possibility of active TB than QFT positive subjects with lower levels of IFN-γ.

2180

Contribution of the T-SPOT.TB assay for detection of latent tuberculous infection (LTBI) in contact tracing procedures

Jean-Paul Janssens[1], Pascale Roux-Lombard[2], Thomas Perneger[3], Marie Metzger[1], Regis Vivien[2], Thierry Rochat[1]. *[1]Pulmonary Diseases, Geneva University Hospital, Geneva, Switzerland; [2]Allergology and Immunology, Geneva University Hospital, Geneva, Switzerland; [3]Social and Preventive Medicine, Geneva University Hospital, Geneva, South Africa*

Setting: Prospective study of subjects screened during contact-tracing procedures, comparing tuberculin skin testing (TST) to a blood assay quantifying IFN-gamma production by lymphocytes exposed to antigens of specific M. tuberculosis complex (T-SPOT.TB).
Methods: 6 different scores of exposure to index case; simultaneous testing by TST and T-SPOT.TB.
Patients: 309 subjects (aged 40±13 years, 52% M, 87% BCG-positive) screened; 73 index cases of active pulmonary TB.
Results: 166 subjects (54%) had LTBI according to TST and 123 (40%) according to T-SPOT.TB; 111 subjects (36%) had both negative TST and T-SPOT.TB; 91 subjects (29%) had both positive TST and T-SPOT.TB; 75 (24%) had a positive TST and negative T-SPOT.TB (97% of them had had a BCG); 32 (10%) had a positive T-SPOT.TB and negative TST. Agreement between TST and T-SPOT.TB (kappa: 0.32) was low. The odds ratios of a positive T-SPOT.TB or TST were moderate for all exposure variables. Among the 6 scores used, exposure was significantly related to result of TST in 3, and to that of T-SPOT.TB in 4. In a 2-way analysis of variance, the number of positive exposure indicators was significantly associated with T-SPOT.TB status (p=0.013), but not with TST status (p=0.15). Indication for treatment for LTBI according to results of either TST or T-SPOT.TB differed in 35% of patients: referring to T-SPOT.TB decreased the number of treatments by 14%; relying on TST alone led to 32 (10%) false negative results.
Conclusion: T-SPOT.TB showed a stronger relationship to scores of exposure than TST; relying on T-SPOT.TB in this setting would decrease the number of treatments for LTBI and the number of false negative results.

198. Pulmonary embolism

2181

Medical treatment in persistent pulmonary hypertension after pulmonary thrombendarterectomy in patients with chronic thrombembolic pulmonary hypertension

Steffen Schiemanck, Gert Hoeffken, Michael Halank. *Department of Respiratory Medicine, University Clinic Dresden, Dresden, Germany*

Introduction: Oral therapy with bosentan in inoperable patients with chronic thrombembolic pulmonary hypertension has shown to improve hemodynamics and exercise tolerance. Some patients do either not improve significantly after pulmonary thrombendarterectomy or deteriorate quickly. Therapeutic options in these patients are limited if no indication for reoperation is to be seen.
Methods: In our pulmonary hypertension unit three patients were evaluated after pulmonary thrombendarterectomy with nonsatisfying long term results for further treatment. 6-minute-walk distance in all patients was below 380 meters. There was no indication for reoperation on the underlying thrombembolic disease. We treated two patients with bosentan and one patient with sildenafil and reevaluated them in a regular follow-up.
Results: The patient on sildenafil and one patient on bosentan improved in maximal oxygen consumption after 9 months of treatment from 11,2 ml/kg body weight/min to 20,7 ml/kg body weight/min and 13,6 ml/kg body weight/min to 16,6 ml/kg body weight/min, respectively. The third patient had less improvement in maximal oxygen consumption from 9,9 ml/kg body weight/min to 10,2 ml/kg body weight/min. Further measurements were undertaken, such as 6-minute-walk-test or proBNP estimation.
Conclusions: Both either bosentan and sildenafil might improve exercise capacity in patients deteriorating after pulmonary thrombendarterectomy who do not have an option for reoperation.

2182

Internal validation of a model to predict the risk of short-term (10 days) adverse outcomes in patients with pulmonary embolism (PE)

Cristina Navarro[1], Aurelio Cayuela[2], Remedios Otero[1], Isabel de Torres[1], Fernando Uresandi[3], David Jimenez[4], Miguel Angel Cabezudo[5], Dolores Nauffal[6], Francisco Conget[7], Elena Laserna[8]. *[1]Service of Pneumology, Hospital Virgen del Rocio, Sevilla, Spain; [2]Research Support Department, Hospital Virgen del Rocio, Sevilla, Spain; [3]Service of Pneumology, Hospital de Cruces, Bilbao, Bizkaia, Spain; [4]Service of Pneumology, Hospital Ramon y Cajal, Madrid, Spain; [5]Service of Pneumology, Hospital Central, Oviedo, Asturias, Spain; [6]Service of Pneumology, Hospital La Fe, Valencia, Spain; [7]Service of Pneumology, Hospital Lozano Blesa, Zaragoza, Spain; [8]Service of Pneumology, Hospital San Juan de Dios, Sevilla, Spain*

Aim: to validate a scale to identify patients with low risk of short-term (10 days) adverse events (death, recurrences and haemorrhages) after an acute Pulmonary Embolism (PE) *Arch Bronconeumol 2005; 41:10*.
Methods: we obtained a predictive scale of adverse events (1). The validation of this scale was carried out in 254 patients diagnosed with PE from February to August of 2005 at 8 Spanish hospitals. The point cut-off was obtained using a ROC curve. The results in the validation sample have been compared with the original derivation sample.
Results: the group of patients diagnosed with PE were 260 patients, 151 women (average age 70.5 years) and 109 men (average age 64.6 years). Six patients (0.2%) were excluded due to mistakes of the basal variables. Finally we could evaluated the predictive model in 254 patients. The area under the ROC curve in the validation sample was 0.73 (95% CI 0.56-0.90). Positive likelihood ratio of 1.90 (95% CI 1.29-2.78) and negative likelihood ratio of 0.30 (95% CI 0.05-1.79).
Conclusion: the predictive model to identified patients diagnosed with PE with low risk of premature adverse events was successfully validated obtained similar results than the original derivation sample.
– (1) Eur. Resp. J. 2005; 26:27s.
– This study is support by grant from ISC III and Pneumology and Thoracic Surgery Society.
– Special thanks to Dr. Monreal, Ms. Hawkins and RIETE register.

2183

Quantitative latex D-dimer outperforms pre-test probability scoring systems in assessment of possible pulmonary embolism

Lutz E. Beckert[1], Claire Taylor[2], Michael Ardagh[2], Martin Than[2]. *[1]Respiratory Medicine, Christchurch Hospital, Christchurch, New Zealand; [2]Emergency Medicine, Christchurch Hospital, Christchurch, New Zealand*

Objective: To compare the performance of Wells pre-test probability score and the D-dimer alone in the assessment of Pulmonary Embolism (PE) in patients presenting to the Emergency Department from the community.
Methods: All patients presenting to the acute care setting with possible PE over a 12 months period were included in the study. Entry points into the study were