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439. New methods to diagnose tuberculosis infection and disease

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Rapid diagnosis of smear-negative tuberculosis by bronchoalveolar-lavage enzyme-linked immunosorbent assay: interim analysis of a TBNET study
 C. Jafari¹, M. Ernst², A. Bossink³, L. Codecasa⁴, A. Strassburg¹, B. Kalsdorf¹, S.F.T. Thijssen³, U. Greinert¹, D. Kirsten⁵, A. Lalvani⁶, D. Goletti⁷, C. Lange¹, on behalf of the European Tuberculosis Network (TBNET). ¹Division of Clinical Infectious Diseases, Research Center Borstel, Borstel, Germany; ²Division of Immunecell Analytics, Research Center Borstel, Borstel, Germany; ³Department of Respiratory Medicine and Tuberculosis, Diaconessenhuis Utrecht, Utrecht, Netherlands; ⁴Villa Marelli Inst. Niguarda Hospital, Niguarda Hospital, Regional Reference Center for Tuberculosis Control, Milan, Italy; ⁵Department of Pulmonary Medicine, Großhansdorf Hospital, Großhansdorf, Germany; ⁶Tuberculosis Immunology Group, Department of Respiratory Medicine, National Heart & Lung Institute, Wright Fleming Institute of Infection & Immunity, Imperial College, London, United Kingdom; ⁷Translational Research Unit, Department of Experimental Research, Istituto Nazionale Malattie Infettive Lazzaro Spallanzani, IRCCS, Rome, Italy

Background: The diagnosis of active pulmonary tuberculosis (pTB) in patients with negative acid-fast bacilli (AFB) smear results from sputum and bronchial secretions is often delayed. We present an interim analysis of an ongoing study of the European Tuberculosis Network (TBNET) on the evaluation of a *Mycobacterium tuberculosis* (MTB) specific ELISpot performed on peripheral blood mononuclear cells (PBMCs) and mononuclear cells from the bronchoalveolar-lavage (BALMCs) for the rapid diagnosis of smear negative pTB.

Methods: Following informed consent, HIV-seronegative patients with a medical history and a pulmonary infiltrate compatible with pulmonary tuberculosis and 3 consecutive negative AFB-smear results from the sputum were prospectively enrolled in the study. In addition to MTB-culture and MTB-specific nucleic acid amplification test (NAT) on sputum/BAL, MTB-specific ELISpot (T-SPOT.TB; Oxford Immunotec, Abingdon, UK) with ESAT-6 and CFP-10 antigens was performed on PBMCs and on BALMCs.

Results: Among 22 patients with pTB 1 patient had a negative ELISpot test result from the BAL. Among 70 patients with an alternative diagnosis, 8 had a positive

ELISpot test result from the BAL. Sensitivity and specificity of the MTB-specific ELISpot performed on BALMCs was 95 percent and 89 percent respectively. In contrast sensitivity and specificity of the MTB specific NAT were 40 percent and 96 percent respectively.

Conclusion: Enumerating MTB-specific T-cells by ELISpot is a promising tool for the rapid diagnosis of smear negative pulmonary tuberculosis.

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RD1-epitopes response correlates with the time since first exposure in close contacts recently exposed to *Mycobacterium tuberculosis*

D. Goletti¹, M.P. Parracino², O. Butera¹, F. Bizzoni¹, S. Carrara¹, D. Vincenti¹, D. Dainotto³, G. Anzidei⁴, E. Girardi². ¹Translational Research Unit, National Institute for Infectious Diseases (INMI) "L. Spallanzani", Rome, Italy; ²Epidemiology Unit, INMI "L. Spallanzani", Rome, Italy; ³Presidio Interzonale di Pneumologia, ASL Roma E, Rome, Italy

Rationale: Tuberculin skin test (TST) has been the only tool used to detect LTBI, but this test is flawed both operationally and with respect to specificity and sensitivity. RD1 based assays are currently under investigation to be evaluated as tools for the diagnosis of LTBI. To assess the potentialities of RD1 based assays in close contacts of patients with smear positive TB, we evaluated the relationship between INF-gamma production in response to a variety of RD1-antigens and the time of exposure to the index case.

Methods and results: In a longitudinal study, 190 recent contacts were enrolled. QuantiFERON-TB Gold (QTF-G) and RD1 selected peptide-based assays were performed. The main outcome of the study was the evaluation of INF-gamma production in response to the assays, expressed as dichotomous (positive/negative) and continuous (IU/mL) measures. In particular INF-gamma production was compared between individuals who were tested within 4 months since first *M. tuberculosis* exposure and individuals who were tested thereafter. Among the 46 TST positive subjects with a close contact to the index case, we found a statistically significant higher INF-gamma production in response to RD1 selected peptides in the contacts exposed to the index case since less than 4 months compared to those exposed a longer time before testing (median value: 2.96 vs 0.3; p=0.01). Conversely no differences were found in response to QTF-G.

Conclusions: These data indicate that among close contacts recently exposed to *M. tuberculosis* the response to RD1 selected peptides assay may be a tool to trace the time of exposure to the index case better than QTF-G.

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Response to RD1 selected epitopes is associated to active tuberculosis. Results of a European multicentre hospital-based study: interim analysis of a European tuberculosis network (TBNET) study

D. Goletti¹, S. Carrara¹, D. Vincenti¹, C. Lange², F. Mengoni³, C. Mastroianni³, D. Cirillo⁴, R. Markova⁵, R. Drenska⁵, M. Amicosante⁶, C. Saltini⁶, E. Girardi⁷. ¹Translational Research Unit, National Institute for Infectious Diseases (INMI) "L. Spallanzani", Rome, Italy; ²Division of Clinical Infectious Diseases Medical Clinic, Research Center Borstel, Borstel, Germany; ³Dipartimento Malattie Infettive e Tropicali, Policlinico Umberto 1° Università "La Sapienza", Rome, Italy; ⁴Patogeni Batterici Emergenti, Istituto Scientifico "San Raffaele", Milan, Italy; ⁵Department of Immunology and Allergology, National Center for Infectious and Parasitic Diseases, Sophia, Bulgaria; ⁶Department of Internal Medicine, University "Tor Vergata", Rome, Italy; ⁷Clinical Epidemiology, INMI "L. Spallanzani", Rome, Italy

There is a clear need for new tools in the diagnosis of active tuberculosis (TB), especially for cases in which microbiological diagnosis is difficult to accomplish such as extra-pulmonary and smear-negative TB. We set-up an INF-gamma assay based on RD1 selected peptides, associated to active TB, differently from those based on RD1 overlapping peptides (QuantiFERON-TB Gold and T-SPOT.TB) able to identify TB infection. Objectives of this study are to evaluate the performance of our assay: 1) on subjects with suspected active TB enrolled in six European hospitals; 2) on patients in which TB diagnosis is difficult to perform; 3) in comparison with commercially available tests for TB infection. Among 414 subjects studied, 188 were diagnosed with active TB and in particular 156 with pulmonary TB, 27 of which with a sputum negative staining, and 32 extra-pulmonary TB; 137 individuals resulted without active TB. Sensitivity for active TB of RD1 selected peptides assay was 72% and specificity was 77%. More than half of these patients (285/414) were simultaneously tested with this assay and one of the two commercially available assays. In this group, sensitivity for active TB disease was 70% for RD1 selected peptides and 86% for the commercial assays, whereas specificity was 78% and 51% respectively.

Based on these data, our assay may be a useful tool in diagnosing active TB, in particular smear negative pulmonary and extra-pulmonary TB in which invasive surgery procedures are often needed to establish a diagnosis.

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Diagnosis of latent tuberculosis infection in patients with chronic liver disease undergoing liver transplantation: performance of the new INF-γ-based assays

M. Losi¹, S. Cerri¹, M. Codeluppi², S. Cocchi², R. D'Amico³, P. Roversi¹, R. Caracciolo¹, M. Meacci⁴, B. Meccugni⁴, F. Rumpianesi⁴, R. Esposito², L. Fabbri¹, L. Richeldi¹. ¹Section of Respiratory Disease, Department of Oncology, Hematology and Respiratory Disease, University of Modena and

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Reggio Emilia, Modena, Italy; ²Section of Infectious Diseases, Department of Medicine, University of Modena and Reggio Emilia, Modena, Italy; ³Section of Statistics, Department of Oncology, Hematology and Respiratory Disease, University of Modena and Reggio Emilia, Modena, Italy; ⁴Microbiology and Virology Laboratory, Policlinico University Hospital, Modena, Italy

Background: Patients with chronic liver disorders undergoing liver transplantation, latently infected with *M. tuberculosis*, are at high risk of active tuberculosis (TB); however, the tuberculin skin test (TST) may be falsely negative due to underlying immunosuppression and concomitant immunosuppressive treatments. These patients also have an increased risk of serious adverse reactions due to preventive treatment with isoniazid. It's important to accurately identify patients with chronic liver disease and latent TB infection (LTBI). The INF- γ -based assays QuantiFERON-TB Gold In tube (QFT-IT) and T-SPOT.TB (TS.TB) represent an improvement over the TST for the diagnosis of LTBI.

Methods: We enrolled in a blinded prospective study 101 patients with chronic liver disease (mean age 54.0 \pm 9.0 years) on a waiting list for liver transplantation; all were simultaneously tested with TST, QFT-IT and TS.TB.

Results: Four patients had test failure for technical reasons and were excluded. At enrolment 19 (19.6%), 30 (30.9%, $p < 0.001$ vs TST) and 23 (23.7%, $p < 0.001$ vs TST) patients were positive with TST, TS.TB and QFT-IT, respectively. TS.TB and QFT-IT showed a good overall agreement ($k = 0.488$). Indeterminate results were more frequent with QFT-IT (8.3%) than with TS.TB (1.0%). Fourteen of 19 TST-positive patients were positive with both INF- γ assays and 4 TST-positive were negative with both blood tests.

Discussion: Based on the results of the INF- γ -based assays, a sizeable fraction of patients with chronic liver disease may have LTBI, partly not recognized by the TST. Preventive treatment of these patients may reduce the risk of active TB.

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T-cell interferon- γ release assay for the diagnosis of tuberculous pleuritis in routine clinical practice

M. Losi¹, L. Codecasa², A. Bossink³, C. Jafari⁴, M. Ernst⁵, S.F.T. Thijsen⁶, D. Cirillo⁷, L. Fabbri¹, L. Richeldi¹, C. Lange⁴, on Behalf of the European Tuberculosis Network (TBNET). ¹Sections of Respiratory Disease, Department of Oncology, Hematology and Respiratory Disease, University of Modena and Reggio Emilia, Modena, Italy; ²Villa Marelli Inst. Niguarda Hospital, Regional Reference Centre for Tuberculosis Control, Milan, Italy; ³Department of Respiratory Medicine and Tuberculosis, Diakonessenhuis Utrecht, Utrecht, Netherlands; ⁴Division of Clinical Infectious Diseases, Research Center Borstel, Borstel, Germany; ⁵Division of Immune-Cell Analytics, Research Center Borstel, Borstel, Germany; ⁶Dept. of Microbiology and Immunology, Diakonessenhuis Utrecht, Utrecht, Netherlands; ⁷Emerging Bacterial Pathogens Unit, S. Raffaele Hospital, Milan, Italy

Introduction: The diagnosis of pleural tuberculosis (pTB) by the analysis of exudative pleural effusions (PE) alone is difficult. In routine clinical practice we evaluated the performance of a commercially available MTB-specific ELISPOT on peripheral blood mononuclear cells (PBMCs) and pleural effusion mononuclear cells (PEMCs) for the diagnosis of pTB.

Methods: MTB-specific ELISPOT (T-SPOT.TB; Oxfordimmunotec, Abingdon, UK) was performed on PBMCs and PEMCs in patients with one-sided exudative pleural effusions and a medical history compatible with pTB at several centers participating in the European tuberculosis network (TBNET).

Results: Eighteen of 20 (90%) patients with pTB tested positive when ELISPOT was performed on PBMCs and 19/20 (95%) when ELISPOT was performed on PEMCs, respectively. Among controls, ELISPOT results were positive in 7/21 patients (33%) when performed on PBMCs (these patients were assumed to be latently infected with MTB). Sixteen of 21 (76%) controls had negative ELISPOT test results performed on PEMCs. The sensitivity and specificity of the T-SPOT.TB for the diagnosis of active pTB was 95% and 76%, respectively.

Discussion: Enumerating MTB-specific T-cells in PBMCs and PEMCs by ELISPOT could be useful for a rapid and accurate diagnosis of pTB in routine clinical practice.

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ESAT-6/CFP-10 and PPD – stimulated cytokine production in patients with MTB infection

R. Drenska¹, R. Markova¹, Y. Todorova¹, V. Tersieva¹, D. Stefanova². ¹Immunology and Allergy, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria; ²Tuberculosis, University Hospital for Tuberculosis and Lung Diseases, Sofia, Bulgaria

Background: Mononuclears, when exposed to mycobacterial antigens produce various cytokines that orchestrate immune responses and are crucial for outcome of the disease. PPD-stimulated cytokine production in tuberculosis patients is well described but less is known about ESAT-6/CFP-10 cytokine responses.

Aim: To evaluate the production of INF- γ , IL-2, IL-6, IL-10 and IL-12, in ESAT-6/CFP-10 and PPD-stimulated whole blood cultures from MTB patients in different stages of disease.

Subjects and Methods: 6 patients with advanced TB (microbiologically confirmed), 5 patients with mildly expressed TB (microbiologically negative), and 5 highly exposed healthy subjects, have been studied. The production of

INF- γ , IL-2, IL-6, IL-10 and IL-12 in response to PPD and ESAT-6/CFP-10 was measured in whole blood culture supernatants by ELISA, according to manufacturer's instruction (Pierce Endogen).

Results: The highest concentrations of INF- γ , IL-2, IL-6, and IL-12 together with the lowest IL-10 were measured in patients with mildly expressed disease. In patients with advanced and confirmed tuberculosis the highest levels of IL-10 were found. In all cases the cytokine production in response to ESAT-6 and CFP-10 was lower than to PPD. IL-12 and IL-10 responses to ESAT-6 and CFP-10 were found very low or not detectable in all patients.

Conclusions: Our preliminary results demonstrate that ESAT-6/CFP-10 antigens induce predominantly the production of proinflammatory cytokines: INF- γ , IL-2 and IL-6. Cytokine responses to ESAT-6/CFP-10 are significantly lower than the PPD – stimulated ones.

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Isoniazid prophylaxis differently modulates T-cell responses to RD1-epitopes in contacts recently exposed to mycobacterium tuberculosis: a pilot study

D. Goletti¹, M.P. Parracino², O. Butera¹, F. Bizzoni¹, S. Carrara¹, D. Vincenti¹, R. Casetti³, D. Dainotto⁴, G. Anzidei⁵, C. Nisii⁶, G. Ippolito⁶, F. Poccia³, E. Girardi². ¹Translational Research Unit, National Institute for Infectious Diseases (INMI) "L. Spallanzani", Rome, Italy; ²Clinical Epidemiology, INMI "L. Spallanzani", Rome, Italy; ³Cellular Immunology, INMI "L. Spallanzani", Rome, Italy; ⁴Presidio Interzonale di Pneumologia, ASL, Roma E, Rome, Italy; ⁵Pediatric Unit, INMI "L. Spallanzani", Rome, Italy; ⁶Epidemiology Unit, Department of Experimental Research, INMI "L. Spallanzani", Rome, Italy

Rationale: Existing data on the effect of treatment of latent tuberculosis infection (LTBI) on T-cell responses to *Mycobacterium tuberculosis* (MTB)-specific antigens are contradictory. Differences in technical aspects of the assays used to detect this response and populations studied might explain some of these discrepancies. In an attempt to find surrogate markers of the effect of LTBI treatment, it would be important to determine whether, among contacts of patients with contagious tuberculosis, therapy for LTBI could cause changes in MTB-specific immune responses to a variety of RD1-antigens.

Methods and results: In a longitudinal study, 44 tuberculin skin test⁺ recent contacts were followed over a 6-month period and divided according to previous exposure to MTB and LTBI treatment. The following tests which evaluate INF- γ responses to RD1 antigens were performed: QuantiFERON-TB Gold, RD1 intact protein- and selected peptide-based assays. Among the 24 contacts without previous exposure that completed therapy, we showed a significant decrease of INF- γ response in all tests employed. The response to RD1 selected peptides was found to be more markedly decreased compared to that to other RD1 antigens. Conversely, no significant changes in the response to RD1 reagents were found in 9 treated subjects with a known previous exposure to MTB and in 11 untreated controls.

Conclusions: These data suggest that the effect of INH prophylaxis on RD1-specific T-cell responses may be different based on the population of subjects enrolled (recent infection versus re-infection) and, to a minor extent, on the reagents used.

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Comparison of Mendel-Mantoux skin test and detection of specific CD4-T-cells with an INF- γ assay in whole blood

U. Mack¹, T. Hodapp², U. Sester², H. Köhler², M. Sester², G.W. Sybrecht¹. ¹Innere Medizin V (Pneumologie), Universitätsklinik des Saarlandes, Homburg, Saar, Germany; ²Innere Medizin IV (Nephrologie), Universitätsklinik des Saarlandes, Homburg, Saar, Germany

The classical Mendel-Mantoux Tuberkulin skin test is increasingly replaced by the measurement of specific T-cells in blood samples. Yet, the correlation of the blood test with the skin test has not been evaluated sufficiently. We compared skin test and whole blood assay for specific CD4 T-cells in 310 patients using purified protein derivative (PPD) and/or ESAT-6 as antigens.

T-lymphocytes from whole blood were stimulated *in vitro* with PPD or ESAT-6 and then measured using a quantitative flow-cytometric whole-blood assay detecting INF- γ - and TNF- α -positive T-cells (Kidney Int. 2004;65:1826).

The use of INF- γ as readout was superior to the use of TNF- α (n=199), as values scattered less widely and showed less false positive results especially in the lower ranges. The skin test correlated well with specific T-cell reaction against PPD (n=310, Spearman $r = 0.5897$, induration in mm against rank, $p < 0.0001$). Measuring T-cell reaction after stimulation with ESAT-6 (n=285), the correlation was slightly weaker (Spearman $r = 0.487$, $p < 0.0001$). The strongest correlation was found when ESAT-6-reactive cells were compared with the rank of the result of PPD-reactive T-cells (Pearson $r = 0.9382$, $p < 0.0001$). A receiver operator characteristic (ROC) analysis revealed that a PPD specific T-cell frequency of 0.10% corresponds to a skin-test induration of 10mm.

Conclusion: The flow-cytometric measurement of specific CD4-T-cells correlates well with the classical skin test. INF- γ is better suited for determination of specific CD4-T-cells than TNF- α .