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39. New methods to diagnose tuberculosis infection and disease

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Comparison of BAL ELISPOT and NAT for the diagnosis of smear-negative tuberculosis – a TBNET study

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Background: The diagnosis of active pulmonary tuberculosis (pTB) in patients (pts) with AFB smear results from sputum and bronchial secretions is often delayed. We present a study of the European Tuberculosis Network (TBNET) on the comparison of a Mycobacterium tuberculosis (MTB) specific ELISPOT performed on cells from the bronchoalveolar-lavage (BALMCs) and from peripheral blood (PBMcs) and MTB-specific nucleic acid amplification technique (NAT) for the rapid diagnosis of smear-negative pTB.

Methods: Following informed consent, pts with a medical history and a pulmonary infiltrate compatible with pTB and negative AFB smear results were prospectively enrolled in the study. MTB-specific NAT on BAL and MTB-specific ELISPOT (T-SPOT.TB, Oxfordimmunotec, Abingdon, UK) on BALMCs and PBMcs were compared.

Results: 2/44 and 3/43 pts with pTB had a negative ELISPOT test result on PBMcs and BALMCs respectively, 41/185 and 103/188 pts with an alternative diagnosis had an ELISPOT+ test result on BALMCs and PBMcs respectively. Among pts with pTB, 27/37 had a negative PCR result from the BAL. Among pts with an alternative diagnosis, 2/127 had a positive PCR result from the BAL. Sensitivity and specificity of the MTB-specific ELISPOT for pTB performed on BALMCs was 92% and 71% and on PBMcs 95% and 43% respectively. In contrast sensitivity and specificity of MTB specific PCR was 27% and 98% respectively.

Conclusion: Enumerating MTB-specific T cells by ELISPOT has a higher sensitivity but lower specificity than MTB-specific PCR for the rapid diagnosis of smear-negative pulmonary tuberculosis

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

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Rationale and objective: To evaluate the performance of an home made IFN- γ assay based on RD1 selected peptides for the diagnosis of active TB in a multicenter European study and to compare the results obtained with TST, QuantiFERON-TB Gold (QF) and T-SPOT.TB (TSPOT).

Methods: Response to RD1 selected peptides was evaluated either by a whole blood assay (WBA), or ELISPOT or by both. In a subgroup of subjects QF and TSPOT were concomitantly evaluated. Correlation with age, origin, sex, BCG vaccination, TB diagnosis (microbiological or clinical), localization [pulmonary (P), extra-pulmonary (EP) or both (P&EP)] and TST was performed.

Results: We analyzed data from 370 subjects enrolled with a suspect of active TB in 7 different European centres. No statistical difference was found in the data obtained among the different centres and by the read-out used (WBA vs ELISPOT). Indeterminate results were found in 12 subjects (3%). Sensitivity for active TB of the RD1 selected peptides test was 73% and specificity 71%. In terms of diagnosis, sensitivity was 72% for microbiological TB and 77% for clinical TB. In terms of localization sensitivity was 73% for P-TB, 75% for EP-TB and 58%

for P&EP-TB. TB diagnosis significantly correlated with origin from country at high TB endemicity, age and BCG vaccination, but not with sex. Comparison with TST, QF and TSPOT showed that sensitivity for active TB was 85%, 78% and 88% respectively, whereas specificity was 51%, 67% and 45% respectively.

Conclusions: Based on these data, our assay may be a useful tool in identifying active TB from those without active disease.

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Rapid diagnosis of tuberculous meningitis by enumeration of cerebrospinal fluid antigen-specific T cells

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Introduction: Rapid diagnostic tests for tuberculous meningitis are a research priority. The enzyme-linked immunospot assay (ELISPOT) detects interferon- γ (IFN- γ) secreting T cells specific for two antigens present in pathogenic *Mycobacterium tuberculosis* complex bacteria and applied to blood has high sensitivity and specificity for the diagnosis of tuberculosis infection, but cannot distinguish between latent and active disease. Antigen-specific T-cells may be concentrated in the cerebrospinal fluid (CSF) in tuberculous meningitis.

Aims and Objectives: To determine whether IFN- γ -secreting *Mycobacterium tuberculosis*-antigen-specific T cells are present in the cerebrospinal fluid of patients with tuberculous meningitis, and to evaluate the feasibility of cerebrospinal fluid ELISPOT for the diagnosis of active tuberculous meningitis.

Methods: We performed a prospective, blinded, hospital-based study of consecutive hospital in-patients with suspected tuberculous meningitis, predominantly in India. IFN- γ -ELISPOT on blood and on CSF was compared with a composite clinical reference standard.

Results: The overnight ELISPOT assay detected *Mycobacterium tuberculosis*-antigen-specific IFN- γ -secreting T cells in cerebrospinal fluid from 9 out of 10 patients with tuberculous meningitis, and 0 out of 7 control patients with non-tuberculous meningitis of other aetiology. This corresponds to a diagnostic sensitivity of 90% (95%CI 56-100%) and specificity of 100% (95%CI 59-100%).

Conclusions: This pilot study demonstrates proof-of-principle for a new T cell-based diagnostic test for tuberculous meningitis, which is rapid, sensitive and specific.

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Performance of blood tests for the diagnosis of latent tuberculosis infection in children

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Background: Performance in high-risk groups of the T-cell interferon-gamma based assays (TIGRA) for the diagnosis of latent tuberculosis infection (LTBI) is a research priority. Younger children represent one of the key high-risk groups since the sensitivity of the tuberculin skin test is low in these subjects due to age-related anergy. TIGRA might perform better than the skin test, also due to the incorporation of a positive control for T-cell activity.

Aim: To evaluate the performance of QuantiFERON-TB Gold (QFT-G), QuantiFERON-TB Gold In-Tube (QFT-IT) and T-SPOT.TB (TS.TB) in paediatric subjects.

Methods: Retrospective analysis of children evaluated for a suspect of either active or latent TB infection over a 3 years period and undergoing testing with at least one TIGRA.

Results: Among 501 children (mean age 11 \pm 5.7 years, range 0-19) indeterminate results due to low positive control were obtained in 12.8% of QFT-G, 4.8% of QFT-IT and 2.4% of TS.TB. Comparing rates of indeterminate tests across different age categories, these were significantly over-represented under the age of 4 for QFT only: 35.9% QFT-G, 20.6% QFT-IT, 1.6% TS.TB. Children with valid results were significantly older for both QFT-G (11.1 vs 5.4 years p>0.001) and QFT-IT (11.9 vs 3.5 years, p<0.001), but not for TS.TB (9.2 vs 7.3 years, p=0.534). For both QFT-G and QFT-IT years of age were positively correlated with the units of interferon-gamma produced in the positive control well.

Conclusions: Younger children have higher rates of indeterminate results when tested with the ELISA-based assays (QFT-G and QFT-IT), as compared to the ELISPOT-based test (TS.TB) for the diagnosis of LTBI.

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Improving T-cell assays for the diagnosis of latent TB infection: potential of a diagnostic test based on IP-10

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Background: We have recently shown that IP-10 is a novel diagnostic biomarker for infection with TB with the potential to improve the performance of the RD1 based T-cell assays.

Methods: Samples were obtained in a cross-sectional study evaluating the risk of infection after TB-exposure in children in Abuja, Nigeria. 59 smear-positive (SS+) contacts, 38 smear-negative culture-positive (SS-) contacts, and 23 community controls (CC) were tested. The production of IP-10 was measured in plasma after stimulation of whole-blood with TB-specific RD1-antigens. The children were classified as IP-10 test positive, negative and indeterminate according to a previously developed test-algorithm. Smear-status of the index-case was used as surrogate-marker for risk of infection. The diagnostic performance of the IP-10 test was compared with the Quantiferon In-Tube (QFT-IT).

Results: In response to TB antigens the median biomarker-concentrations in the SS+, SS- and CC groups were 1358 pg/ml (IQR 278-2535 pg/ml), 117 pg/ml (32-341 pg/ml), and 149 pg/ml (25-497 pg/ml) for IP-10; and 106 pg/ml (16-585 pg/ml), 1pg/ml (0-3 pg/ml), and 0 pg/ml (0-6 pg/ml) for IFN- γ . The IP-10 test was positive in 71% of SS+, 21% of SS- and in 26% CC; compared to 71% SS+, 8% SS- and 13% CC with the QFT-IT. There was excellent agreement between the IP-10 and QFT-IT tests (78%, kappa=0.64) and a strong trend in positivity rate with the sputum smear grade of the index case (p<0.0001).

Conclusions: IP-10 is expressed high amounts and has comparable diagnostic performance to IFN- γ . IP-10 holds promise for the development of a new generation of tests with increased sensitivity and simpler read-out formats.

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Memory response to RD1 antigens in TST+ subjects: a better tool to detect LTBI?

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Introduction and objective: Short-incubation IGRAs are currently used for the screening of individuals for latent tuberculosis (LTBI). Aim of this study is to improve the detection of LTBI in TST+ subjects by evaluation, in response to *M. tuberculosis* antigens, of: a) secretion of cytokines different from IFN- γ ; b) memory response.

Methods: TST+ healthy subjects were studied using Quantiferon-TB Gold in tube (QFT) test and a whole blood home made assay (WBA) based on PPD and RD1 proteins. WBA responses were evaluated at day 1 (effector response) and day 7 (memory response) by the detection of IFN- γ (by ELISA), IL-2, TNF- α (by FACS analysis).

Results: Among the 52 TST+ subjects studied (38 of them were BCG-vaccinated), at day 1 after in vitro stimulation, IFN- γ response was found in 31/52 to QF (60%), 51/52 (98%) to PPD and 30/52 (58%) to RD1 proteins. To note that among the QF responders 30/31 were RD1 proteins positive. In the "QF-responders" a significant amount of IFN- γ , IL-2 and TNF- α was found at day 1 in response to PPD and RD1 proteins. Conversely the "QF-responders" individuals were IFN- γ negative in response to RD1 proteins at day 1; however an IFN- γ memory response was recovered in 4/22 (18%) to RD1 proteins. Interestingly in these memory-responders, RD1-specific-IL-2 and -TNF- α detection was already found at day 1.

Conclusions: Among TST+ subjects, QF may help to identify more likely LTBI subjects. IFN- γ memory response to RD1 proteins and other cytokines different from IFN- γ (TNF- α and IL-2) detected at day 1 may improve LTBI identification. Whether TST+ persons with positive memory response to RD1 proteins but negative to QF are at risk for the development of TB needs to be elucidated.

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IFN-gamma response on T-cell based assays in HIV patients for detection of tuberculosis infection

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Background: Determination of IFN- γ for the detection of tuberculosis infection

with *Quantiferon-TB GOLD In Tube (QFT-G-IT)* and *T-SPOT.TB (TS.TB)* in HIV patients, comparing the results with tuberculin skin test (TST).

Materials and methods: We enrolled 46 HIV infected patients, 9 of them with a CD4 cell number less than 250 cel/ μ l. Eleven patients had a BCG scar and for five BCG status was unknown.

Results: *TS.TB* and *QFT-G-IT* were positive in 19.6% (9/46) and 28.3% (13/46), otherwise, TST was positive (>5mm) in 37% (17/46). Differences between both T-cell assays were not significant. Concordance between *TS.TB* and *QFT-G-IT* was 83.72% ($\kappa=0.562$). Concordance of *TS.TB* and *QFT-G-IT* with TST was 76.74% ($\kappa=0.470$) and 83.72% ($\kappa=0.641$), respectively. We obtained one indeterminate result with *QFT-G-IT*, that was invalid for *TS.TB* because there were not enough cells for the assay. In this case, TST was negative and the patient had CD4 less than 250 cel/ μ l. In this group, *TS.TB*, *QFT-G-IT* and TST were positive only once. In non-BCG vaccinated patients *TS.TB* was positive in 10% (3/30), *QFT-G-IT* in 26.7% (8/30) and TST in 23.3% (7/30), whereas, in BCG vaccinated patients *TS.TB* was positive in 36.4% (4/11), *QFT-G-IT* in 27.3% (3/11) and TST in 72.7% (8/11).

Conclusions: 1. Although we obtained more positive results with *QFT-G-IT* with regard to *TS.TB* in the detection of tuberculosis infection in HIV patients, the results were not significant.

2. In patients with CD4 less than 250 cel/ μ l, the sensitivity and the concordance of IFN- γ tests were the same that with TST.

3. Both *in vitro* assays seem to have lower interference with the BCG than TST.

4. Performance of these *in vitro* assays permit to detect anergy.

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Evaluating the potential of IP-10 and MCP-2 as biomarkers for the diagnosis of infection with mycobacterium tuberculosis

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Background: We have identified the chemokines IP-10/CXCL10 and MCP-2/CCL8 as potential diagnostic biomarkers for infection with *Mt* in line with the Interferon- γ Release Assay. The aim of the study was to evaluate the potential of diagnostic tests based on IP-10 and MCP-2, and to compare the performance with the Quantiferon- γ In-Tube (QFT-IT) test.

Methods and findings: IP-10 and MCP-2 were determined in supernatants from whole blood stimulated with *Mt*. specific antigens. Samples were obtained from 80 patients with culture and/or PCR proven TB, and 124 unexposed healthy controls. TB patients produced significantly higher levels of IP-10 (median 2158pg/ml) and MCP-2 (median 379pg/ml) compared with IFN- γ (median 215pg/ml, p<0.0001). ROC curve analysis revealed that the area under the curve was similar for IFN- γ (0.98) and IP-10 (0.95, p=0.1), but lower for MCP-2 (0.89, p<0.04). We established IP-10 and MCP-2 diagnostic tests based on ROC curve analysis and predefined criteria. The QFT-IT, IP-10 and MCP-2 test were positive in 81%, 83% and 71% of the TB patients, and 0%, 7% and 1% of the controls, and agreement between tests was high >89% (kappa >0.77). Mitogen responses were impaired in TB patients probably due to immunosuppression. To compensate for this, we evaluated a mitogen-adjusted cut-off, which significantly increased the proportion of IP-10 test positive TB patients to 95%.

Conclusion: IP-10 and MCP-2 are potent diagnostic biomarkers with equivalent performance to IFN- γ . As MCP-2 and IP-10 are induced in high amounts they could be utilized for in-vitro tests with operational advantages and improved sensitivity in immune-compromised patients.