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Risk for progression to tuberculosis among arriving immigrants with a positive IGRA

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Background: The incidence of tuberculosis (TB) in The Netherlands is partly driven by disease progression of unidentified TB infection (LTBI) in immigrants. Screening newly arriving immigrants for LTBI with the QuantiFERON®-TB Gold In-Tube (QFT-GIT) might be a promising intervention.
Aims and objectives: To estimate the risk for progression to active tuberculosis within two years of entry in newly arriving immigrants with a positive QFT-GIT response.
Methods: In a case-base design, we determined the prevalence of positive QFT-GIT responses among a random sample of 1375 immigrants newly arrived during 2009-2011 (the base). Active tuberculosis patients (cases) within two years after entry were extracted from the Netherlands Tuberculosis Register from a cohort of immigrants arriving in 2006. We assumed a QFT-GIT sensitivity of 90% and no transmission occurred after entry.
Results: Among the 1369 immigrants with valid QFT-GIT responses, overall 20% were positive. Stratified by low (<100/100.000), middle (100-200/100.000) and high (≥200/100.000) tuberculosis incidences in person's region of origin, QFT-GIT positivity was 16%, 24% and 26%, respectively. The risk for progression to disease per 100,000 newly arriving immigrants from low, middle and high incidence regions was 347 (95% CI: 75-1185), 398 (86-1355), and 801 (132-2942), respectively, if QFT-GIT positive, compared to a risk of disease of 7 (0-113), 14 (0-212), and 31 (0-491) per 100,000 if QFT-GIT negative.
Conclusions: Newly arriving immigrants with a positive QFT-GIT response have a significant risk of progression to TB within two years of entry, even immigrants from low incidence regions. QFT-GIT offers added-value in the immigration screening program.

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Head-to-head analysis of IGRAs and skin-testing in immunocompromised patients: Interim analysis of a multicenter TBNET study

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The superior sensitivity of IFNγ release assays (IGRAs) in diagnosing LTBI above tuberculin skin testing (TST) may be compromised by immunodeficiency. We performed direct comparisons between tests in immunocompromised patients.
IGRAs (T-SPOT/QFT) and TST were performed and clinical data on drugs and TB exposure were collected. Interim results from 193 organ transplant patients and 204 patients with rheumatoid arthritis (RA) are presented.
In transplant patients, 80% had results for all 3 tests, which were less often positive for TST (11.7%) than for T-SPOT (20.8%) and QFT (15.6%, p=0.018). Agreement was substantial between IGRAs (κ=0.61), and only fair between IGRAs and TST (κ=0.22, T-SPOT; 0.34, QFT). All 3 tests were more often negative despite TB risk factors in patients <1 year post transplant as compared to patients ≥1 year. In RA patients, 91% had result for all 3 tests, which were positive in 36.0% (TST), 26.3% (T-SPOT), and 24.2% (QFT, p=0.002). Agreement was only fair between IGRAs and TST (κ=0.29, T-SPOT; 0.33, QFT) and substantial between IGRAs (κ=0.77). Negativity rate despite TB risk factors was high in all tests, particularly when receiving >3 immunomodulatory drugs. All tests in both groups were associated with TB-exposure without marked confounding by age, sex, number of drugs or time post transplant.
Despite TB risk factors, results are more often negative in transplant patients in the first year post transplant and in RA patients with >3 immunomodulatory drugs, likely due to higher level of immunosuppression. This emphasises the need for LTBI screening prior to transplantation or immunosuppressive treatment to increase diagnostic accuracy.

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Specific diagnosis of tuberculosis infection sent via mail – IGRAs on paper

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Objectives: IFN-γ release assays (IGRAs) are the most accurate diagnostic tests for latent TB. IP-10 is a chemokine expressed in concert with IFN-γ, but in >100 fold higher levels.
The aim of this study was to investigate if IP-10 in plasma from M.tuberculosis specific antigen stimulated whole blood dried and stored on filter paper was stable and could be used for TB diagnosis.
Methods: In Spain, whole blood from 78 patients with culture confirmed TB, and 109 healthy controls were subjected to Quantiferon (QFT) testing. Following, IP-10 was measured in plasma supernatant using ELISA and 25µ aliquots were dried on Whatman903 filter paper.
Filter paper was sent by normal mail to Denmark where 2 discs were cut from the filter paper using a normal office 6mm hole punch. The amount of IP-10 in filter paper was measured using ELISA.
Results: Median P-10 levels in TB patients were 1,073pg/ml and 27.3ng/ml in filter paper and plasma; and in controls 44pg/ml and 1.0ng/ml, respectively (p<0.0001 for both). The correlation between filter paper and plasma IP-10 was very high (r2=0.93). The Area Under the ROC curve was comparable 0.89-0.91 for both IP-10 tests and IFN-γ and the 3 tests had comparable sensitivity and specificity 74-78% and 100-98%, respectively (all n.s. differences)
Conclusion: Plasma from M.tb. antigen stimulated blood can be dried on filter paper and transported over long distances at ambient temperature before analysis. Compared to the currently available IGRAs, the filter paper version allows for high throughput centralized analysis, and could increase the dissemination of specific tests for LTBI in resource restraint settings where IGRAs as we know them today are too complicated to do.

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Smoking is a strong independent risk factor for indeterminate and false negative IGRA results

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Objectives: 10-40% of IGRA results are indeterminate or false negative. HIV infection, old age and immunosuppressive treatment are known risk factors, but in many cases the reason remains unknown.
The aim of this study was to identify risk factors for indeterminate and false negative TB results.
Materials and methods: 80 HIV-positive and 112 HIV-negative patients with bacteriologically confirmed pulmonary TB were included and tested with the QuantiFERON-TB (QFT-IT) and a Luminex based IP-10 test. By multivariate analysis the following parameters were tested as risk factors for an indeterminate or false negative QFT-IT or IP-10 test result: HIV-infection, age>50 years, smoking and alcohol consumption.
Results: Smokers (28/192) had a higher risk of an indeterminate or false negative result by both the QFT-IT (OR 7.1, CI: 2.2-23.0) and the IP-10 test (OR 11.2, CI: 3.3-37.8). ORs for HIV-infection was 3.6 (CI: 1.8-7.5) and 5.3 (CI: 2.2-11.8) respectively.
Antigen levels were lower in smokers for both IFN-γ (median 22 vs. 79.5 pg/ml, p<0.01) and IP-10 (median 939.4 vs. 2446.2, p<0.01).
Positivity rate (incl. indeterminate results) was lower for smokers by both the

QFT-IT (50 vs. 74%, p=0.03) and the IP-10 test (42 vs. 76, p<0.01). These findings could be reproduced in HIV-positive and –negative patients individually and smoking and HIV-infection were independent risk factors (p=1.00).
Apart from HIV-infection no other parameter tested was identified as a risk factor for a false negative or indeterminate result by either test.
Conclusion: Smoking increases the risk of having a false negative or indeterminate IGRA results. IGRA test results should be interpreted with care in smokers.

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Bifunctional T cells allow for discrimination between latent and active tuberculosis
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Introduction: The diagnosis of active tuberculosis remains challenging especially in a high tuberculosis incidence country as South Africa. We propose that further knowledge on the Mycobacterium tuberculosis (Mtb)-specific bifunctional cytokine immune response will help to immunologically distinguish latent versus active disease.
Material and methods: Tuberculosis was diagnosed by culture positivity. Latent tuberculosis was confirmed by a tuberculin skin test on people who did not have clinical symptoms of active tuberculosis. Following informed consent, peripheral blood was drawn from 12 persons with active and 12 persons with latent tuberculosis. To compare the Mtb-specific immune response, 200.000 mononuclear cells/well were stimulated with the Mtb-specific antigens ESAT6/CFP10, MTP65 and PPD overnight and Interferon gamma (IFNg), Interleukin (IL)-2 or IFNg/IL-2 producing T cells were detected as spot forming cells in a FluoroSpot plate.
Results: As expected, Mtb-specific IFNg immune response did not discriminate between active and latent tuberculosis. In contrast persons with latent tuberculosis had significantly higher numbers of MTP65-specific cells producing IL-2 (p=0.035) and bifunctional cells producing IFNg/IL-2 (p=0.03). Furthermore an algorithm using the ESAT6/CFP10 induced IFNg, IL-2 and double positive cells significantly distinguished between the two cohorts (p=0.001).
Conclusion: These preliminary data reveal that defining effector and central memory T cells might allow a better differentiation between latent and active tuberculosis in a blood cell test even in a high incidence country. This is an ongoing study.

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IP-10 measurement using a point of care test for the diagnosis of active tuberculosis
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Introduction: Measurement of IP-10 released by whole blood stimulation with tuberculosis (TB) specific antigens has recently been proposed as a new tool for the diagnosis of tuberculosis (TB) with a sensitivity (SE) similar to that of the Quantiferon TB in tube-assay (QFT, Cellestis). More data is needed on the detection as well as indeterminate rates in IP-10-dependent diagnosis for active TB.
Methods: IP-10 was measured in the supernatants of QFT tests in 81 patients with known TB status (active TB at the time of sampling in 25 patients). QFT results were indeterminate in 41 patients, negative in 13, and positive in 27 of the 81 patients. IP-10 was measured by using a multiplex assay (MPA, Biorad) (50 patients only), or by using a semiquantitative lateral flow immunoassay (LFI, Milenia Biotec). Samples with a mitogen control $\geq$500 pg of IP-10 per mL were considered determinate. The cut-off for IP-10 positivity in determinate tests was 455 pg/mL both MPA and LFI.
Results: Indeterminate results were reduced by 56% (18/41) using IP-10 LFI in comparison with QFT. 5% (2/40) determinate QFT results turned indeterminate in IP-10 LFI. Pearson correlation for IP-10 measurements by MPA and LFI was 0.53 (95%CI 0.3-0.7). The SE in patients with active TB was 56% for QFT (14/25, CI 0,35-0.76), 70% for IP-10 MPA (19/25; CI 0.55-0.9) and 63% for IP-10 LFI (15/24; CI 0.4-0.81).
Conclusion: IP-10 measured by LFI and IFN-γ measured by QFT had comparable detection and indeterminate rates. A combination of IFN-γ and IP-10 might increase SE by lowering indeterminate rates. Point of care tests as the IP-10 LFI, which correlated fairly well with IP-10 MPA, should be further evaluated for the diagnosis of TB infection.

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Methylated HBHA produced in *M. smegmatis* discriminates between active and non-active TB disease among the QFT-IT-responders
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Background: Challenge in tuberculosis (TB) research is to develop a test to distinguish, among the responders to an IFN-γ release assay (IGRA), those who control *M. tuberculosis* (Mtb) replication from those that cannot. IFN-γ response to the Heparin-binding-hemagglutinin (HBHA) of Mtb has been associated with latent TB infection (LTBI), but the cumbersome procedures to purify the methylated and immunological active form of the protein from Mtb or *M. bovis* BCG have prevented the implementation of a diagnostic test. Aim of this study was to evaluate the IFN-γ response to methylated HBHA of Mtb produced in *M. smegmatis* (rHBHAms) in subjects at different stages of TB who scored positive to QuantiFERON-TB Gold in-tube (QFT-IT).
Methodology/Principal findings: 87 subjects at different stages of TB who scored positive to QFT-IT were selected. IFN-γ response to *in vitro* whole blood stimulation with rHBHAms was evaluated by short-term and long-term tests and detected by ELISA or flow cytometry. We demonstrated that the IFN-γ response to rHBHAms is mediated by $CD4^+$ T-cells with an effector-memory phenotype. This response, evaluated also by short-term-tests is significantly lower in active TB than in remote LTBI (p=0.0010), past TB (p=0.0152) and recent infection (p=0.05). These results were confirmed by long-term tests and by qualitative analysis using ROC analysis.
Conclusions: For the first time to our knowledge, we showed that the T-cell response to a recombinant and methylated HBHA of Mtb produced in *M. smegmatis* in a whole blood system is immunogenic and potentially useful to discriminate between active and non-active TB disease among those responsive to QFT-IT.