

Comparison of QuantiFERON®TB Gold with tuberculin skin test to improve diagnostics and routine screening for tuberculosis infection among newly arrived asylum seekers to Norway

Brita Askeland Winje

Master of Public Health

MPH 2008:15



Nordic School of Public Health



Master of Public Health – Examensarbete –

Examensarbetets titel och undertitel Sammenligning av QuantiFERON®TB Gold med tuberkulin hudtest for å forbedre diagnostikk og rutinemessig screening for tuberkulosesmitte blant nyankomne asylsøkere til Norge				
Författare Brita Askeland Winje				
Författarens befattning och adress Rådgiver, Avdeling for infeksjonsovervåking, Folkehelseinstituttet, Postboks 4404, Nydalen, 0403 Oslo, Norge				
Datum då examensarbetet godkändes 2008-09-05			Handledare NHV/extern Max Petzold/Einar Haldal	
Antal sidor 43	Språk – examensarbete Engelsk	Språk – sammanfattning Norsk	ISSN-nummer 1104-5701	ISBN-nummer 978-91-85721-53-5

Sammanfattning

Introduksjon: QuantiFERON®TB Gold (QFT) er en ny blodtest for påvisning av tuberkulosesmitte, men med få data så langt fra undersøkelse av immigranter. Målet med studien var å sammenligne resultat av QFT og tuberkulin hudtest blant nyankomne asylsøkere i Norge og å vurdere hvilken rolle QFT bør ha i screening for latent tuberkulose.

Metode: Alle asylsøkere, 18 år eller eldre, som ankom Tanum asylmottak fra september 2005 ble invitert til å delta og ble inkludert etter informert samtykke. Inkludering pågikk inntil et forhåndsbestemt antall på 1000 inkluderte ble nådd. Siste deltager ble inkludert i juni 2006. Deltagelse innebar en QFT test og standardiserte spørsmål, i tillegg til den lovpålagte tuberkulintesten og lungerøntgen.

Resultat: Totalt 2813 asylsøkere ankom Tanum asylmottak i inkluderingsperioden (sept 05-juni 06). Blant de 1000 deltagerne hadde 912 gyldige testresultater og ble inkludert i analysen, 29 % (264) hadde positiv QFT, mens 50 % (460) hadde positiv tuberkulintest (indurasjon ≥ 6 mm). Det indikerer en høy andel smittede personer i denne gruppen. Blant deltagerne med positiv tuberkulintest hadde 50 % negativ QFT, mens 7 % av dem med negativ tuberkulintest hadde positiv QFT. Det var en signifikant sammenheng mellom økning i tuberkulinutslag og sannsynligheten for å ha positiv QFT. Samsvar mellom testene var 71-79%, avhengig av grenseverdi for tuberkulin. Det var bedre samsvar mellom testene for ikke-vaksinerte personer.

Konklusjon: Ved å implementere QFT som rutine kan videre oppfølging avsluttes for 42% av dem som ville ha blitt henvist basert kun på tuberkulinresultat (≥ 6 mm). Andelen som henvises vil være den samme enten QFT implementeres som erstatning for eller som supplement for å bekrefte en positiv tuberkulinreaksjon, men antallet som testes vil variere mye. Ulike tilnærminger vil identifisere samme andel (88-89%) av asylsøkere med positiv QFT og/eller sterkt positiv tuberkulinutslag (≥ 15 mm), men ulike grupper vil mistes.

Nyckelord

Tuberkulose, screening, tuberkulin hudtest, QuantiFERON®TB Gold



Master of Public Health

– Thesis –

Title and subtitle of the thesis				
Comparison of QuantiFERON®TB Gold with tuberculin skin test to improve diagnostics and routine screening for tuberculosis infection among newly arrived asylum seekers to Norway				
Author Brita Askeland Winje				
Author's position and address Adviser, Norwegian Institute of Public Health, Department of Infectious Disease Epidemiology, P.O.Box 4404, Nydalen, 0403 Oslo, Norway				
Date of approval September 5, 2008			Supervisor NHV/External Max Petzold/Einar Heldal	
No of pages 43	Language – thesis English	Language – abstract Norwegian/English	ISSN-no 1104-5701	ISBN-no 978-91-85721-53-5

Abstract

Introduction: QuantiFERON®TB Gold (QFT), a new blood test that detects tuberculosis infection, currently provides few data from immigrant screening. This study aimed to compare results of QFT and tuberculin skin tests (TST) among newly arrived asylum seekers in Norway and also assess the role of QFT in screening for latent tuberculosis.

Methods: All asylum seekers, 18 years or older, who arrived at Tanum reception center from September 2005 were invited to participate and included after informed consent. Enrollment was continued until a fixed sample size of 1000 participants was reached. The last participant was included in June 2006. In addition to mandatory TST and chest X-ray, study participants underwent a QFT test and answered standardized questions.

Results: A total of 2813 asylum seekers arrived at Tanum reception center during the inclusion period. Among the 1000 study participants, 912 showed valid test results and were included in analysis; 29% (264) had a positive QFT test and 50% (460) tested positive with TST (indurations ≥ 6 mm), indicating a high proportion of latent infection within this population. Among the TST-positive participants, 50% were QFT-negative, whereas 7% of the TST-negative participants were QFT-positive. A significant association occurred between increase in size of TST induration and positive QFT result. Test agreement (71%–79%) depended on the chosen TST cut-off and was higher for nonvaccinated individuals.

Conclusions: By implementing QFT as a routine screening test further follow up can be avoided for 42% of asylum seekers who would have been referred based only on a positive TST (≥ 6 mm). The proportion of individuals referred remained the same whether QFT replaced TST or confirmed a positive TST; however, the number of individuals tested varied greatly. Different approaches would identify the same proportion (88%–89%) of asylum seekers with either a positive QFT or a strongly positive TST (≥ 15 mm), but different groups will be missed.

Key words

Tuberculosis, screening, tuberculin skin test, QuantiFERON®TB Gold

Table of content

Introduction	3
Tuberculosis	3
Epidemiology	3
Tuberculosis in Norway	4
The Norwegian Tuberculosis Control Programme.....	6
Diagnostic tests.....	8
Tuberculin skin test	8
Interferon-Gamma Release Assays (IGRA).....	9
 Aim	 10
Methods	11
Design and setting	11
Ethical considerations.....	12
Diagnostic tests.....	12
Data handling and statistical analyses	13
Results	14
Enrolment and characteristics of the study group	14
Representativity of the study group.....	16
Results from tuberculin skin test and QuantiFERON®TB Gold	16
Agreement between QFT and TST.....	19
Predictors of positive tests.....	19
Implications for screening	20
Discussion.....	21
Agreement between QFT and TST.....	22
Possible explanations for discordant results.....	23
Predictors of positive tests.....	24
Implications for screening in Norway	25
Strengths and weaknesses of the study.....	26
Bias	26
Random error.....	26
Systematic error.....	27
Assessment of external validity.....	29
Conclusions	30
Acknowledgements	30
References	32
Attachments.....	38

Introduction

Tuberculosis

Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis* or less frequent of other species within the *M. tuberculosis*-complex. Tuberculosis can affect all organs, but it most commonly affects the lungs. Pulmonary tuberculosis is of most concern as normally only persons with pulmonary tuberculosis are infectious. The bacterium is transmitted via airborne droplets from the throat and lungs of people with the active pulmonary disease which are then inhaled by others. Left untreated, each person with active tuberculosis disease will infect on average between 10 and 15 people every year (1).

A person infected with *Mycobacterium tuberculosis* will not necessarily become sick with the disease. In most infected persons the immune system “walls off” the bacteria and protected by a thick, waxy coat the bacteria remains in the body and can lie dormant for years, as latent tuberculosis infection. A person with latent tuberculosis infection is healthy, has no symptoms and cannot transmit the disease to others. While the risk of acquiring infection is largely exogenous in nature, determined by the characteristics of the source case, environment and duration of exposure, the risk of progression from infection to disease is largely endogenous, determined by the integrity of the cellular immune system. The lifetime risk of progression from latent infection to disease is estimated to be 5-10% with highest risk the first years following infection (1). When the immune system is weakened the chances of becoming sick are greater. People with hiv-infection or other immune-suppressive diseases have a much higher risk of active disease if infected with tuberculosis. Weakening of the immune system later in life (as with older age, malign disease etc) may cause reactivation of latent infection many years after the person became infected.

Tuberculosis can be cured with a six months regimen with combined drugs. Also resistant bacteria can normally be treated with drugs, but the treatment is longer and associated with more side effects.

Epidemiology

Globally tuberculosis remains a major threat to public health. According to World Health Organization (WHO) there were 8.8 million new cases of tuberculosis disease in 2005 and 1.6 million people died from tuberculosis world wide. Tuberculosis is a disease of the poor and more than 80% of the cases appear in 22 high-burden, low income countries. The epidemic mainly affects young adults in their most productive years (2). The incidence rate of tuberculosis and drug resistant tuberculosis has increased over the past 15-20 years, fuelled by the hiv-epidemic, poor access to diagnostic and

treatment facilities, uneven access to drugs and unfavourable treatment outcome. In 2005 the incidence rate of tuberculosis was stable or decreasing in all six WHO regions, but due to population growth the number of new cases is still increasing in Asia and Africa. Emergence of resistant strains poses a serious and growing global health problem and has reached alarming rates in some areas.

Someone in the world is newly infected with tuberculosis every second and WHO has estimated that one third of the world population is currently infected with *M. tuberculosis* (2). The enormous pool of persons with latent tuberculosis infection serves as reservoir for new cases and challenges control of tuberculosis worldwide. The prevalence of latent infection differs for different regions. In 1997 WHO estimated the prevalence of latent tuberculosis infection to be 35% in Africa, 44% in Southeast Asia and 15% for Europe (3). The increase in travelling and migration, increase in number of foreign workers and close neighbouring countries with alarming rates of drug resistant tuberculosis, makes tuberculosis control remain a public health concern in Norway and in other Nordic and European low-prevalence countries. Compared to the rest of the world the tuberculosis epidemiology in the Nordic countries is favourable. All Nordic countries have experienced a reduction in the total annual rate of notified cases of tuberculosis over the past decades and are regarded as low-incidence countries for tuberculosis. During 1975 until 2005 the incidence rate of tuberculosis in Sweden decreased from 17.6 to 6.3 per 100 000 population, in Denmark from 12.2 to 7.8, in Norway from 12.5 to 6.3, in Iceland from 18.3 to 3.7 and in Finland from 74.2 to 6.9 cases per 100 000 (4, 5). Even with the similarities in the crude incidence rate in the Nordic countries there are differences in the proportion of foreign-born cases due to differences in immigration. In 2005 the proportion of foreign born among notified cases of tuberculosis was 73% in Sweden, 61% in Denmark, 78% in Norway and 64% in Iceland, while the situation is different in Finland where only 10% were foreign-born. Tuberculosis represents a manifest problem among the foreign-born population in the Nordic countries as in most other European countries, despite the lower proportion seen in Finland (4, 5).

Tuberculosis in Norway

Notification of tuberculosis in Norway

Norway has an overall low incidence rate of tuberculosis with 6.3 cases per 100 000 population in 2006, while the incidence rate remains high in risk groups. The transmission rate is low and an increasing proportion of cases occurs among immigrants from countries with high prevalence of tuberculosis (Figure 1). The incidence rate was highest among African-born persons with 326 per 100 000 population followed by Asia with 69 per 100 000 population in 2006 (6-9).

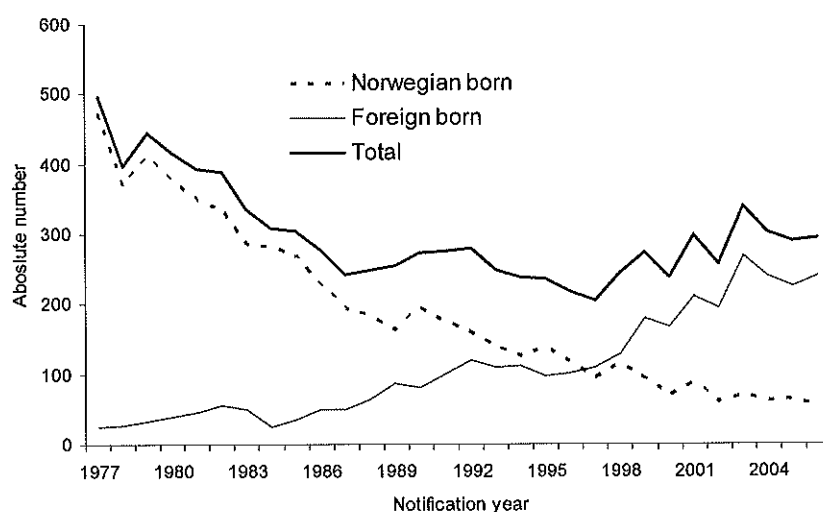


Figure 1 Data from the Norwegian tuberculosis register on notification of tuberculosis in Norway 1997-2006 by origin (6)

Tuberculosis in the Norwegian-born population is rare. The incidence rate decreased from 1.9 to 1.3 per 100 000 population during the six year period from 2001-2006. The incidence rate for foreign-born patients is high and increased in the same period from 62.2 to 69.6 per 100 000 population. Foreign-born persons count for an increasing proportion of notifications of tuberculosis in Norway. The proportion has increased from 29% in 1990 to 81% in 2006.

Transmission of tuberculosis in Norway

Genotyping, RFLP fingerprinting (Restricted Fragment Length Polymorphism), has been performed routinely on all *M. tuberculosis* isolates in Norway since 1994. The genotyping shows that almost 80% of the patients with culture confirmed tuberculosis in Norway have a unique strain (10). This indicates that most cases of tuberculosis are due to imported new strains rather than ongoing transmission of tuberculosis in Norway. Data from the 12-year period from 1994-2005 show that increase in immigration to Norway led to increase in incidence of tuberculosis during the period, but otherwise had little effect on the transmission of tuberculosis within the country (11). A study examined the long term risk of tuberculosis among non-Nordic immigrants to Norway from 1986-2002. Only few immigrants were diagnosed with tuberculosis disease on arrival and most foreign-born patients developed the disease several years post-migration. The rates for different population groups were 7 to 90 times higher than the crude tuberculosis incidence for Norway and applied to both genders, pulmonary and extra-pulmonary sites (12).

The Norwegian Tuberculosis Control Programme

The aim of public health is to reduce the disease burden and promote health for the whole population. Interventions are often divided into efforts with the aim of promoting health and of preventing disease. Controlling infectious diseases has been a cornerstone in public health activities for several decades. Key elements in tuberculosis control are early diagnosis and correct treatment of tuberculosis disease. This will cure the individual patient, interrupt transmission of tuberculosis to others and prevent the bacteria from becoming drug resistant. In countries with a low prevalence of tuberculosis, including the Nordic countries, preventive treatment of infected persons is a useful supplement (13). In Norway most cases of tuberculosis are caused by reactivation of latent infection. Identification of persons with latent tuberculosis infection, especially those at highest risk of progression to disease, and wider use of preventive treatment is necessary to reduce the incidence of tuberculosis in the future. Screening for tuberculosis is therefore an important element in the Norwegian tuberculosis control programme.

Screening is a secondary preventive measure. The aim is to contribute toward a more successful cure, less suffering and better health than when symptoms alone prompt diagnosis. Screening was defined in 1951 by the US Commission on Chronic Illness as;

“the presumptive identification of unrecognized disease or defect by the application of tests, examinations or other procedures which can be applied rapidly. Screening tests sort out apparently well persons who probably have a disease from those who probably do not. A screening test is not intended to be diagnostic. Persons with positive or suspicious findings must be refereed to their physicians for diagnosis and necessary treatment” (14).

Wilson and Junger listed criteria to guide the rational development of screening programs in 1968 (Text box 1).

The criteria for screening by Wilson and Junger (1968)

1. *The condition sought should be an important health problem*
2. *There should be an accepted treatment for patients with recognized disease*
3. *Facilities for diagnosis and treatment should be available*
4. *There should be a recognizable latent or early symptomatic stage*
5. *There should be a suitable test or examination*
6. *The test should be acceptable to the population*
7. *The natural history of the disease, including latent disease, should be adequately understood*
8. *There should be an agreed policy on whom to treat*
9. *The cost of case-finding should be economically balanced in relation to possible expenditure on medical care as a whole*
10. *Case-finding should be a continuing process and not “once for all” project*

(From: Concepts of epidemiology by Raj Bhopal, Oxford University Press 2002, page 147)

Screening for tuberculosis have two aims; (1) detect infectious tuberculosis patients to offer treatment and eliminate further transmission, and (2) detect persons with latent tuberculosis infection to offer preventive treatment (15, 16). In Norway, the following groups are obliged to undergo screening for tuberculosis:

1. Persons from countries with a high prevalence of tuberculosis who will be spending more than three months in Norway, and refugees and asylum seekers.
2. Persons who arrive from, or who have spent at least three months in a country with a high prevalence of tuberculosis, and who will be taking up or resuming duties in health and social services, in teaching positions or in other positions connected with child care.
3. Other persons whom there is a medical suspicion that they are, or have been, at risk of being infected (16).

High prevalence countries for tuberculosis are listed in the current guidelines and on the website for the Norwegian Institute of Public Health (www.fhi.no). The countries listed are mainly in accordance with countries where WHO has estimated an incidence rate of >50 per 100 000 population. Screening for tuberculosis includes a tuberculin skin test and also chest x-ray for certain groups. The preliminary screening is conducted in the municipalities. The aim is to identify persons at risk of being infected and refer them to hospitals for further medical evaluation. A specialist in infectious- or lungdiseases is responsible for the decision to offer treatment or not. Asylum seekers and other refugees are a high priority group for the screening.

Prescription of preventive treatment is still limited, despite the screening programme and the current recommendations. There are probably several reasons, but the lack of an accurate diagnostic tool for diagnosis of latent infection is one main concern. Culture of a specimen with identification of species within the *M. tuberculosis*-complex is the gold standard for diagnosis of tuberculosis disease. It is referred to as a confirmed diagnosis. There is no gold standard for the diagnosis of latent infection. Whereas a vast array of molecular and immunologic tools are available to diagnose many infectious diseases, detection of latent tuberculosis infection still relies on the century old tuberculin skin test (TST). The TST has been widely used as a screening test and has contributed substantial epidemiological information on infection trends and on the magnitude of the disease, but has well known limitations. The main concern is that considerable cross reactivity exists. This causes a high proportion of false positive results in regard to tuberculosis infection. According to the Wilson and Junger criteria for a screening programme, a test used in screening should be fairly cheap, easy to use and acceptable to the public. For the understanding of tuberculosis epidemiology, it is of great importance to use tools that enables us to obtain knowledge of the true prevalence of infection and on interactions between the tuberculosis infection and the disease. Vast resources are spent on this screening program. The screening is initiated from the health authorities and it is mandatory for risk groups for tuberculosis to undergo screening. Therefore it is a public health responsibility to evaluate and improve screening performance. Even though the extent and organization of screening program differs within the Nordic countries, all suffer from the poor specificity of the tuberculin skin test. There is an ongoing debate in many countries on best practice for implementation

of the new diagnostics and the study is relevant for many countries, including other Nordic countries.

Diagnostic tests

Sensitivity and specificity of a test is defined as the proportion of persons correctly identified with or without a given characteristic respectively. The positive and negative predictive values refers to the probability that a person with a positive test is a true positive meaning that he/she does have the disease (positive predictive value) or is a true negative meaning he/she does not have the disease if the test is negative (negative predictive value) (14). The positive and negative predictive values depend on the prevalence of the condition in the population tested. A test is sufficiently reliable if it gives replicable results and is sufficiently valid if it detects most people with the target disorder (high sensitivity) and excludes most people without the disorder (high specificity) and if a positive test usually indicates that the disorder is present (high positive predictive value) (1).

Tuberculin skin test

Purified Protein Derivate (PPD) tuberculin is the most commonly used tuberculin. It is isolated from culture filtrate by protein precipitation. The reaction to intracutaneously injected tuberculin is the classic example of a delayed type hypersensitivity (DTH) reaction. Infection by *M. tuberculosis* usually results in a DTH response to tuberculin that is detectable 2-12 weeks after infection occurred. The most important mediating cells involved are the CD4- and CD8- T-lymphocytes, as well as monocytes and macrophages. The induration is recorded in millimetres and is measured as the size of a bump under the skin 72 hours after the test was applied (15, 17).

The sensitivity of the test is in general estimated to be high in immune-competent individuals and approaches 100% for persons with latent infection and normal immune responses, while false negative results occur in patients with advanced tuberculosis disease or severe immune-suppression (18-22). There is an association between the increase in size of the TST induration and the likelihood of true tuberculosis infection and a TST > 15mm has been interpreted as reflecting true latent infection (23-25). While the sensitivity of the test is fairly well characterized, the specificity of the test remains unpredictable (1). The *Mycobacterium Bovis*-BCG used in BCG-vaccination (Bacillus Calmette-Guérin) and a wide spectre of non-tuberculosis mycobacteria contain proteins analogous to those of *M. tuberculosis* and considerable cross reactivity exists. Thus, a positive test may reflect infection with various non-tuberculosis mycobacteria or previous BCG-vaccination.

There is a vivid debate over appropriate cut-off values for TST. As sensitivity and specificity are interdependent, a gain in the sensitivity of a test is offset by a loss of

specificity and vice versa. Over the last two decades a Bayesian approach to the interpretation of TST has replaced rigid criteria for interpretation of test results, taking into account the probability of a result reflecting true infection. Guidelines for tuberculin testing have therefore adopted different cut-off values for different risk categories.

The TST is cheap, safe and requires no laboratory services. The drawbacks are the many disturbing factors in the interpretation of a TST result. Variability in performance and interpretation of the test has shown to be significant and two visits to health care facilities are required for administration and interpretation of the test. Also, repeated testing can cause boosted reactions. The fact that both false positive and false negative results occur may cause unnecessary treatment or lack of indicated treatment. The high proportion of false positive results is a main challenge as this demands great resources from the health care system and also causes unnecessary concern among the affected persons. The current use of the tuberculin skin test is under debate as there is an urgent need for a more specific test for detection of latent infection. This is important both on individual and public health level.

Interferon-Gamma Release Assays (IGRA)

More specific immunological tests based on cellular production of interferon- γ in response to the *M. tuberculosis* specific antigens ESAT-6 and CFP-10 have recently been developed for the diagnosis of tuberculosis infection. These protein antigens used in the assays are absent in all vaccine strains of *M. bovis*-BCG and most non-tuberculosis mycobacteria (NTM), except *M. marinum*, *M. zulgai*, and *M. kansasii*. These tests can therefore diagnose infection with *M. tuberculosis* with a higher specificity (26, 27). There are two assays commercially available on the market, the QuantiFERON® TB Gold (QFT) (Cellestis, Ltd, Carnegie, Australia) and the T-SPOT-TB (Oxford Immunotec Ltd, Oxford, UK). The same antigens are involved in both assays, although they do not measure the same immunological response. The QuantiFERON® TB Gold offers logistic advantages and may be suitable for routine screening.

As there is no gold standard for latent infection estimation of sensitivity is studied (1) among patients with active tuberculosis disease, (2) among exposed persons (categorized into gradients of exposure), and (3) as concordance of IGRA and TST. Differences in design and populations make comparisons between studies difficult.

Available research evidence on IGRA sensitivity suggests that both TST and QFT have suboptimal, but similar sensitivity among patients with newly diagnosed, active tuberculosis (28-31). In patients with active disease the T-SPOT-TB assay was somehow superior to the other tests (32). The assays have been tested in numerous contact investigations and the IGRA seem to correlate better with exposure to *M. tuberculosis* than tuberculin and distinguish more accurately between infections caused by tuberculosis from infections with non-tuberculosis mycobacteria and BCG-*bovis* (27,

28, 33-36). It is a concern that IGRA might be less sensitive than TST for detecting latent infection among immune-competent persons and sensitivity measurements have been reported in the range of 75-97% dependent on the study population and design (37-39). Among persons with immune-suppression the IGRA seem to have higher sensitivity than TST for detection of latent infection and only severe immune-suppression may lead to indeterminate or false negative results (40-43).

Specificity has been estimated from studies of healthy persons with a very low likelihood of exposure. It is reported to be excellent in the range from 98-99% as neither IGRA is affected by BCG-vaccination and most non-tuberculosis mycobacteria (31, 44). The first study of the predictive value of QFT for the development of active tuberculosis disease was recently published. The study of contacts of smear positive patients in Germany suggests that QFT determines more accurately than TST the presence of latent infection with at least equivalent sensitivity for predicting progression to tuberculosis disease. The high rate of progression to active tuberculosis of those QFT-positives (14,6%), compared to TST-positives (2,3%) has significant health and economic implications for tuberculosis control (45).

Most studies have been conducted in low-prevalence countries. Studies of IGRA in high-prevalence settings are few and results vary. One study suggests 80% prevalence of latent infection in urban India, while another reports 56% prevalence among healthy adults in South Africa (46, 47).

Data are limited for immigrant screening and the few studies are small. Among 21 studies of QFT in screening programs, only two studies are published on the performance of QFT in immigrant screening, one of them only in Japanese. The other study included 100 immigrants from high prevalence countries attending an outpatient clinic in Italy, where 44% and 15% were positive with TST (≥ 10 mm) and QFT, respectively (48). The remaining studies were mostly related to screening of health care workers. There is a need for more data on the performance of the assays in routine screening of immigrants from countries with high prevalence of tuberculosis.

There is an ongoing debate in Norway and in other Nordic and western countries on how to implement the new diagnostics in a setting with routine screening and what effect different approaches will have on the screening programme

Aim

This study is part of a more extensive research project to assess the role of preventive treatment in Norwegian tuberculosis control.

The aim of the present study was to improve diagnostics and routine screening for latent tuberculosis infection among newly arrived asylum seekers in Norway.

Specific objectives:

- compare results of QuantiFERON®TB Gold and tuberculin skin test results among newly arrived asylum seekers by measuring the agreement for different cut-off values for TST, origin and previous BCG-vaccination,
- identify and compare predictors for positive QFT and TST results, and
- to assess the effect on the screening programme for different approaches of implementing QFT in routine screening for tuberculosis infection, by measuring the proportion referred, case detection rate and false positive rate for different cut off values of TST.

Methods

Design and setting

The number of asylum seekers arriving in the Nordic countries as in Norway has changed considerably over time and will continue to change based on the current political commitment and the global disaster situation. In 2005 and 2006 the number of asylum seekers applying for residency in Norway was approximately 5300 persons (49). Independent of the number of arrivals the coverage of screening of asylum seekers is high. Approximately 95% of all asylum seekers arriving in Norway are housed in one central reception centre, Tanum, the first days after arrival. Legal issues, immediate medical needs and screening for tuberculosis are taken care of before the asylum seekers are transferred to more long term facilities elsewhere in Norway.

All asylum seekers, 18 years or older, arriving at Tanum reception centre from the study started in September 2005 were consecutively invited to participate in the study and were included if they gave informed consent (attachment 1 and 2). Enrolment in the study was continued until a fixed sample size of 1000 participants was reached. Inclusion of the sample size of 1000 participants defined the study period and the last participant was included in June 2006.

The large sample size of 1000 participants was chosen to: (1) give reasonable number as basis for conclusions, especially for discordant results of negative TST and positive QFT and positive TST (>15mm) and negative QFT respectively, as these groups would be most affected by different approaches for implementation of QFT in routine screening, (2) limit the influence of random errors and (3) it was a pragmatic approach as the funding for the study was limited to 1000 tests. Direct power calculation for sample size was not possible as there were no pilot data, or reasonable estimates, available for concordance of test results for this group. Other studies had small sample sizes and mainly included persons with positive skin tests.

All information material was translated into 12 different languages and was available as written leaflets and on CD for illiterate asylum seekers. Translators should be obtained

from the community if necessary. The participants were invited to the study the day after arrival in Norway, at the time the tuberculin skin test was applied. The signed consent form was collected and the blood sample was drawn when the asylum seekers returned for reading of their Mantoux-test after three days. This gave asylum seekers sufficient time to decide upon participation. Asylum seekers are regarded as a vulnerable group when they arrive in a country applying for asylum and ethical issues were assessed carefully. Persons younger than 18 years were not invited to participate for two reasons; (1) at the time when the study started the QFT had not been tested in this group and there were no data available on how to interpret results, (2) informed consent is complicated for under aged persons, especially in the asylum seeker group as they often arrive without legal parents. The only exclusion criteria for enrolment were young age and lack of informed consent.

Consenting participants had a QFT test, questions regarding demographics, previous BCG-vaccination, previous history or known exposure to tuberculosis, in addition to mandatory TST and chest x-ray. Observed presence of a scar was considered evidence of BCG-vaccination, and known contact with an infectious case of tuberculosis was reported as exposure. The blood sample for QFT was drawn at the time the TST was read. Data were available for all asylum seekers only on country of origin and sex. In order to assess the representativity of the study group additional data on age and TST-results were collected from the mandatory screening of a consecutive sample of 250 persons who did not consent to participate in the study: 125 persons at study start and 125 persons half way through the study.

Ethical considerations

Normal procedures in accordance with national guidelines were followed, regardless of participation in the study. The Regional Ethics Committee for Medical research recommended the study (S-05122) and the Norwegian Data Inspectorate gave permission (23145).

Diagnostic tests

TST was performed according to the Mantoux method with Purified Protein Derivate (PPD): RT 23, 2 TU from SSI, Copenhagen, Denmark. Four experienced nurses applied and read the test. A test was considered positive if the induration was ≥ 6 mm after 72 hours. Reading was double if the indurations were large, showed adverse reactions or were hard to read.

QuantiFERON®TB Gold in-tube-test (Cellestis Ltd, Carnegie, Victoria, Australia) was used. One ml of venous blood was drawn into one tube pre-coated with synthetic peptide antigens and one tube without antigens (negative control sample) and transported the same day to the Norwegian Institute of Public Health for analysis.

Samples were incubated, processed and stored in accordance with the manufacturer's instructions before harvested plasma was subjected to Enzyme-Linked Immunosorbent Assay (ELISA) analysis, including IFN- γ standard for quantification. The quality of all laboratory analyses and calculation of the results was controlled by using the accompanying QFT analysis software. A sample was considered positive if exceeding the standard cut-off value at 0.35 IU IFN- γ /ml.

A positive result on the QFT would potentially increase the likelihood of preventive treatment. Therefore all positive results were confirmed by re-analysis of the same plasma sample before reported as positive. In lack of a confirmatory positive test, the QFT result was reported as non-conclusive and the participant excluded from the study. Medical follow-up of the participant was then in accordance with current guidelines based on TST and chest X-ray.

Data handling and statistical analyses

All data was entered into Excel in standardised format by one responsible nurse at the reception centre. An updated Excel file was sent to the Norwegian Institute of Public Health every month for assessment of data quality and data completeness. When the study period was completed, a file with the QFT results from the laboratory was matched with the Excel file, to double check entry of blood test results. In addition, all information entered on every fifth participant throughout the study was manually checked against medical records at the reception centre. Data entry was also double checked for all participants with discrepant results as: negative TST and positive QFT or TST ≥ 15 mm and negative QFT results. Only participants with a valid QFT result and a recorded TST result were included in statistical analysis.

Kappa-statistics, 95% confidence intervals for single samples (Wilson's method) and unpaired samples (Newcombe's method) were calculated in Confidence Interval Analysis Software (CIA – Trevor N Bryant, 2000) (50). The other statistical analyses were performed in STATA 9.2 (Statacorp, Texas, 77845, USA). Four different uni- and multivariate unconditional logistic regression analyses were performed in order to identify predictors for positive QFT and TST results. The outcome variables were QFT result and TST result ≥ 6 mm, ≥ 10 mm and ≥ 15 mm. Age (in 4 groups using 10 year periods), sex, origin (Europe, Asia, Africa), BCG-vaccination (scar/no scar), previous history of tuberculosis (yes/no) and known exposure (yes/no) were included as independent variables in a preliminary multivariable regression analysis. The independent categorical variables were expressed as dummy variables. The Spearman correlation coefficient was used to check the correlation between all pairs of independent variables. We subtracted one variable at a time using the likelihood ratio test as elimination criterion ($p < 0.05$). The same approach was used to test the significance of the two-way interaction terms between the independent variables in the final model. The odds ratios calculated from the estimated coefficients in the final models were used to measure the strength of association.

Results

Enrolment and characteristics of the study group

The time required to have the fixed sample size of 1000 asylum seekers consenting to participation, defined the study period. The study lasted from September 2005 until June 2006. A total of 2963 asylum seekers, 18 years and older, arrived in Norway during this period and 2813 (95%) of them entered Tanum reception centre and were invited to participate. Among them 1813 (64%) declined participation.

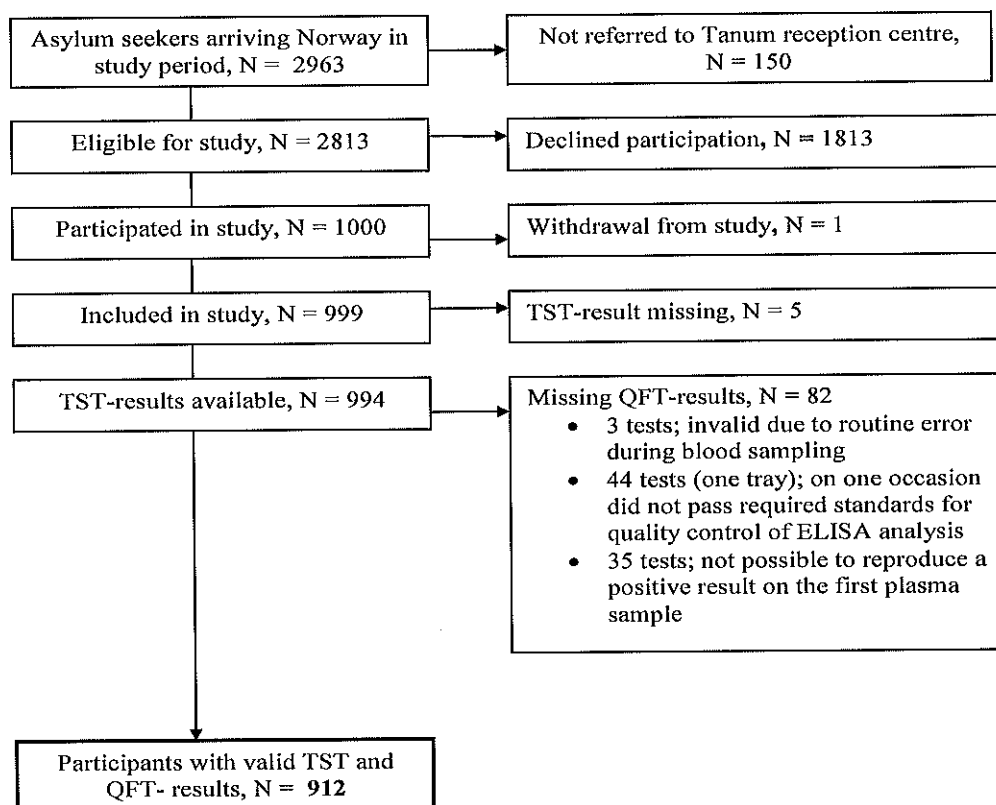


Figure 2 Asylum seekers 18 years or older, entering Norway in the study period and those included in the study group.

Of the 1000 participants enrolled, 88 (8.8%) were later excluded (Figure 2). One participant withdrew from the study after inclusion and five were excluded since a TST result was not recorded. Routine error during blood sampling (blood was sampled only in tubes without antigens) caused exclusion of another three participants. On one occasion (one tray containing 44 tests) the ELISA analysis performed in the laboratory did not pass the internal technical control requirements defined by the manufacturer for

the test (linearity of standard curve). This was due technical mistakes done in the laboratory. It was not feasible to collect new blood samples and the 44 participants were excluded from the study. For 35 cases exclusion was attributable to the requirement in the study to have positive results confirmed by a second analysis on the same plasma sample. In one case the first analysis showed too high background value in the negative control sample, while the second analysis was positive. There was not enough plasma for a new analysis and this case (1) was excluded due to lack of confirmation of the positive result. The remaining cases (34) were excluded due to a negative test result in the confirmatory analysis. Many of these cases had QFT results clustered at or very near the IFN- γ cut-off value (0.35 U/ml). Their TST indurations were in the range of 0-22 mm (median 7 mm).

This left 912 participants with valid QFT- and TST results. The study group included significantly more men and fewer persons from Europe compared to all arriving asylum seekers (Table 1).

Table 1 Distribution by sex and origin of all asylum seekers, 18 years or older, who arrived at Tanum reception centre between September 19th 2005 and June 12th 2006 and of those participating in the study.

	Total arrivals at Tanum			Study group		
	No.	%	95% CI	No.	%	95% CI
Total	2813	100		912	100	
Sex		% male			% male	
male	1983	70.5	68.8 - 72.2	685	75.1	72.2 - 77.8
female	830			227		
Origin						
Europe	473	16.8	15.5 - 18.2	115	12.6	10.6 - 14.9
Asia*	1304	46.3	44.5 - 48.2	418	45.8	42.6 - 49.1
Africa	1021	36.3	34.5 - 38.1	371	40.7	37.5 - 44.0
America	7	0.2	0.1 - 0.5	4	0.4	0.2 - 1.1
Unknown	8	0.3	0.1 - 0.6	4	0.4	0.2 - 1.1

*Includes Turkey (no=15)

Median age was 29 years (range 18-67) and the asylum seekers arrived from 60 different countries. Main countries of origin were Iraq (168), Somalia (155), Russia (61), Iran (63), Eritrea (59) and Afghanistan (47), representing 61% of the study group. Russian and Serbian origin dominated among the European participants. Among those with African origin, 342 (92%) came from Sub-Saharan Africa. Scar from previous BCG-vaccination was observed in 658 (72%) participants, more frequently among Europeans (87%) and less frequently among Africans (65%).

Representativity of the study group

By comparing 250 non-participants with the study group, we found no significant differences between the groups on important variables as size of the TST, presence of BCG scar or age distribution, while more men were included and fewer asylum seekers of European origin participated (Table 2). The reversed bias found among the study group and all arrivals in the study period and the study group and the group of non-participants is as expected.

Table 2 Characteristics of the 912 asylum seekers included in the study and 250 non-participants

		Study group			Control group		
		No.	%	95% CI	No.	%	95% CI
Total		912			250		
Sex		% male			% male		
	<i>male</i>	685	75.1	72.2 - 77.8	161	64.4	58.3 - 70.1
	<i>female</i>	227			89		
Origin							
	<i>Europe</i>	115	12.6	10.6 - 14.9	51	20.4	15.9 - 25.8
	<i>Asia*</i>	418	45.8	42.6 - 49.1	113	45.2	39.1 - 51.4
	<i>Africa</i>	371	40.7	37.5 - 44.0	83	33.2	27.7 - 39.3
	<i>America</i>	4	0.4	0.2 - 1.1	2	0.8	0.2 - 2.9
	<i>Unknown</i>	4	0.4	0.2 - 1.1	1	0.4	0.1 - 2.2
Age							
	<i>18-29 years</i>	471	51.6	48.4 - 54.9	130	52	45.8 - 58.1
	<i>30-39 years</i>	291	31.9	29.0 - 35.0	76	30.4	25.0 - 36.4
	<i>40-49 years</i>	115	12.6	10.6 - 14.9	34	13.6	9.9 - 18.4
	<i>50+ years**</i>	35	3.8	2.4 - 4.8	10	4	2.2 - 7.2
BCG-status							
	<i>no scar</i>	246	27.0	24.2 - 29.9	64	25.6	20.6 - 31.4
	<i>scar</i>	658	72.1	69.2 - 75.0	168	67.2	61.2 - 72.2
	<i>unknown</i>	8	0.9	0.4 - 1.7	18	7.2	4.6 - 11.1
TST-induration							
	<i>0-5mm</i>	452	49.6	46.3 - 52.8	138	55.2	49.0 - 61.2
	<i>6-9mm</i>	149	16.3	14.1 - 18.9	48	19.2	14.8 - 24.5
	<i>10-14mm</i>	170	18.6	16.2 - 21.3	38	15.2	11.3 - 20.2
	<i>15mm+</i>	141	15.5	13.3 - 18.0	26	10.4	7.2 - 14.8

*Includes Turkey (no=15)

** Only 4 participants were older than 60 years, the oldest 67

Results from tuberculin skin test and QuantiFERON®TB Gold

A total of 460 (50%) participants tested positive with TST (≥ 6 mm) while 264 (29%) tested positive with QFT. Among the TST positive participants 50% were QFT

negative, whereas 7% of the TST negative participants (< 6 mm) tested positive with QFT (Table 3).

Table 3 Results of QuantiFERON®TB Gold and tuberculin skin test (> 6mm) among 912 asylum seekers included in the study, by origin, , sex, BCG-status and age

	No. Included	Positive QFT-result total No. % (95% CI)		Positive TST > 6mm No. % (95%CI)	
Total	912	264	29 (26.0 - 31.9)	460	50 (47.2 - 53.7)
Origin					
<i>Europe</i>	115	22	19 (11.9 - 26.3)	60	52 (43.1 - 61.1)
<i>Asia</i>	418	82	20 (15.8 - 23.4)	165	39 (34.9 - 44.2)
<i>Africa</i>	371	159	43 (37.8 - 47.8)	235	63 (58.3 - 68.1)
<i>America</i>	4	1	25		
<i>Unknown</i>	4	0			
Sex					
<i>Male</i>	685	202	29 (26.1 - 32.9)	336	49 (45.3 - 52.8)
<i>Female</i>	227	62	27 (21.3 - 33.1)	124	55 (48.1 - 61.1)
BCG-scar					
<i>No scar</i>	246	68	28 (22.1 - 33.2)	113	46 (39.8 - 52.2)
<i>Scar</i>	658	196	30 (26.3 - 33.2)	343	52 (48.3 - 55.9)
<i>Unknown</i>	8	0		4	50
Age					
<i>18-29 years</i>	471	129	27 (23.4 - 31.4)	204	43 (38.9 - 47.8)
<i>30-39 years</i>	291	75	26 (20.7 - 30.8)	158	54 (48.6 - 59.9)
<i>40-49 years</i>	115	40	35 (26.1 - 43.5)	74	64 (55.3 - 72.5)
<i>50 years+</i>	35	20	57 (40.7 - 73.5)	24	69 (52.0 - 81.4)

Significantly more participants tested positive on TST than QFT for all groups, except the age group older than 50 years. Participants 18-29 years were less frequently positive on TST than the older age groups. Positive TST and QFT tests were most common among African participants (63% and 43%). European participants more often tested positive on TST (52%) than Asian participants (39%), while QFT-results were similar among European and Asian participants, 19% vs 20% respectively (Table 3).

As illustrated in figure 3a positive QFT results was reported for all sizes of TST indurations, but more frequently with increase in the size of the TST indurations. Figure 3b illustrates the clear association between the proportion of positive QFT and increase in the size of the TST indurations.

Figure 3a.

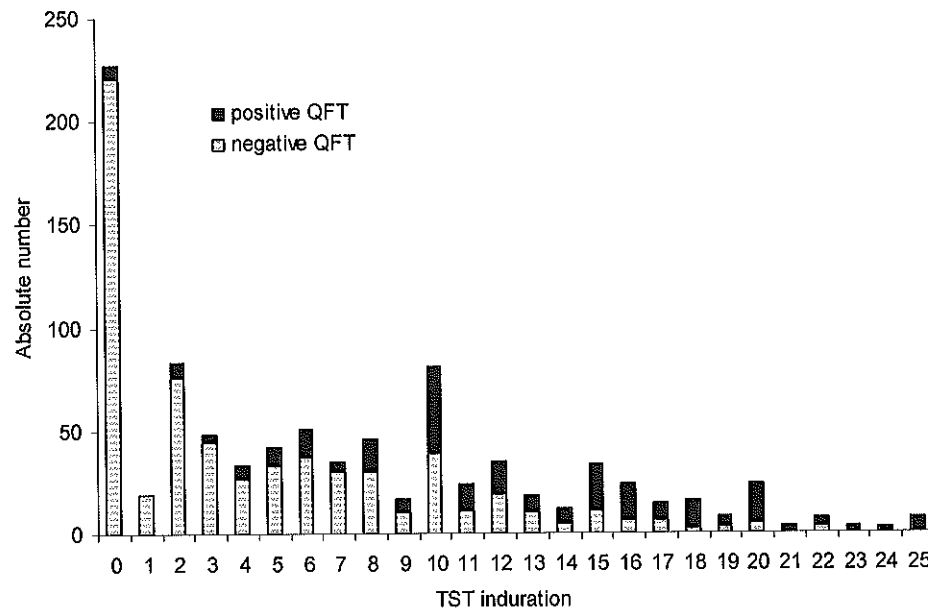


Figure 3b.

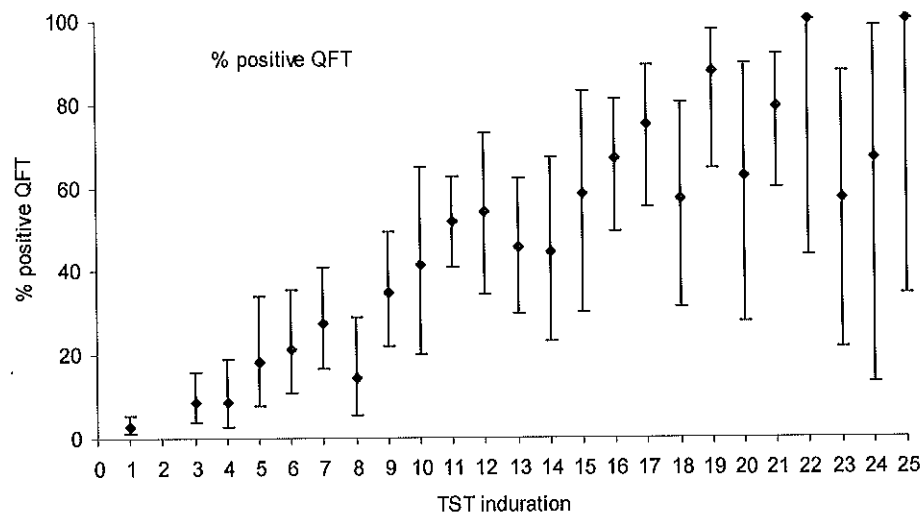


Figure 3a and b QuantiFERON®TB Gold (QFT) results for each millimetre tuberculin skin test induration in absolute numbers (Fig 3a) and as percentage of positive QFT tests with 95% CI (Fig 3b), among 912 asylum seekers included in the study

Among the 1000 asylum seekers initially enrolled in the study, five were diagnosed with culture-confirmed tuberculosis as outcome of the screening. Out of these, two were among participants later excluded from the study; one (TST 15 mm) did not have a QFT-result and one (TST 17 mm) only had a single positive QFT, without a confirmatory test done. Among the remaining three, one patient (TST 9 mm) had a negative QFT result and two were QFT positive (10 and 18 mm TST). All five reported exposure to and symptoms of tuberculosis and had chest x-rays indicative of tuberculosis.

Agreement between QFT and TST

Overall kappa-values for agreement between QFT and TST were fair to moderate depending on the chosen cut-off values for TST, and they were highest with TST cut-off ≥ 10 mm (Table 4).

Table 4 Agreement between tuberculin skin test and QuantiFERON®TB Gold among 912 asylum seekers included in the study by different cut-off values of tuberculin skin test indurations.

TST	QFT	TST ≥ 6 mm No. (%)	TST ≥ 10 mm No. (%)	TST ≥ 15 mm No. (%)
Positive	Positive	232 (25)	190 (21)	104 (11)
Negative	Negative	420 (46)	527 (58)	611 (67)
Positive	Negative	228 (25)	121 (13)	37 (4)
Negative	Positive	32 (4)	74 (8)	160 (18)
Total agreement		652 (72)	717 (79)	715 (78)
Kappa (95% CI)		0.43 (0.37 - 0.49)	0.51 (0.45 - 0.57)	0.39 (0.32 - 0.47)

The agreement (TST ≥ 10 mm) was higher among participants with no scar from BCG: Kappa 0.66 (95% CI 0.56 - 0.77) compared to vaccinated participants: Kappa 0.45 (95% CI 0.37 - 0.52). The agreement was lower for European participants: Kappa 0.32 (95% CI 0.18 - 0.52) than for African: Kappa 0.45 (95% CI 0.36 - 0.54) and Asian: Kappa 0.58 (95% CI 0.48 - 0.67) participants.

Predictors of positive tests

Predictors of a positive QFT in multivariate regression analysis were age, origin and exposure (Table 5). With TST ≥ 15 mm as outcome variable gender, age, and origin were predictors.

Table 5 Results from multivariate regression analyses with positive QuantiFERON®TB Gold and tuberculin skin test reaction ≥ 15 mm as outcome variables among 912 newly arrived asylum seekers included in the study.

Variabel	Characteristic	Total No.	QFT			TST ≥ 15 mm		
			<i>a</i> OR (95%CI)	p-value	p-value variable	<i>a</i> OR (95%CI)	p-value	p-value variable
Sex	<i>male</i>	685				1		0.001
	<i>female</i>	227				1.9 (1.3-2.8)	0.001	
Agegroup	<i>18-29 years</i>	471	1		0.001	1		0.002
	<i>30-39 years</i>	291	1.1 (0.8-1.5)	0.649		1.5 (1.0-2.3)	0.082	
	<i>40-49 years</i>	115	1.8 (1.1-2.9)	0.013		1.9 (1.1-3.3)	0.025	
	<i>50+ years</i>	35	4.6 (2.2-9.5)	0.001		4.3 (1.9-9.5)	0.001	
Origin	<i>Asia</i>	418	1		0.001	1		0.001
	<i>Europe</i>	115	1.0 (0.6-1.7)	0.901		2.4 (1.3-4.5)	0.005	
	<i>Africa</i>	371	3.3 (2.4-4.6)	0.001		3.8 (2.0-4-6)	0.001	
Exposure	<i>no</i>	898	1		0.019			
	<i>yes</i>	14	4.0 (1.3-12.9)	0.019				

Blanks in multivariate analyses means that the variable was not a significant predictor of a positive test in the final model of the respective test (either TST > 15 mm or a QFT). Participants with American or unknown origin were excluded from statistical analysis, due to low number of cases.

Adjusting TST cut-off to ≥ 10 mm did not change the predictors, while only age and origin were significant with 6 mm cut-off. Presence of BCG-scar was not a significant predictor for either test, regardless of TST cut-off value. As persons reporting previous history of tuberculosis also reported known exposure these variables were strongly correlated (Spearman's rho 0.657, $p < 0.001$) and did not fit into the same model.

Implications for screening

There is no gold standard for latent infection. For the purpose of discussing different screening approaches we have chosen to define latent tuberculosis infection as having a positive QFT regardless of TST induration or a TST ≥ 15 mm with a negative QFT. In this study 301 participants are defined as having latent tuberculosis infection; the 264 QFT positive participants and the 37 with a TST ≥ 15 mm and a negative QFT (Table 6). If QFT is implemented in routine screening the number in need of referral could be reduced by 42% compared with referral based only on a positive TST ≥ 6 mm (50% TST positives compared to 29% QFT positives)

Table 6 The impact of different approaches to routine screening with tuberculin skin test and QuantiFERON®TB Gold on the proportion referred for treatment, proportion of persons with latent infection detected and proportion non-infected referred for treatment

Screening programme approach	Referral criteria	Proportion (%) referred for treatment	Proportion (%) LTBI* cases detected (detection rate)	Proportion (%) non-infected referred for treatment (false positive rate)
Only TST	TST $\geq$ 6 mm	50	89	42
	TST $\geq$ 10 mm	34	75	27
	TST $\geq$ 15 mm	15	47	0
Only QFT	QFT positive	29	88	0
Two step (first TST, then QFT)	TST $\geq$ 6 mm and QFT positive, or TST $\geq$ 15 mm	29	89	0
	TST $\geq$ 10 mm and QFT positive or TST $\geq$ 15 mm	25	75	0
	TST $\geq$ 15 mm (with or without QFT positives afterwards)	15	47	0

**Latent infection is defined as either having a positive QuantiFERON(R)TB Gold test (264 participants) or a tuberculin skin test $\geq$ 15 mm and a negative QuantiFERON(R)TB Gold test (37 participants).*

The table shows the effect on screening for different approaches based on identification of 301 cases of latent tuberculosis infection.

The proportion referred is less affected by whether QFT is implemented as a replacement of or as a supplement to TST. Different approaches would identify the same percentage (88-89%) with a positive QFT and/or a TST $\geq$ 15 mm, but the number of tests required will vary. Changing the TST cut-off value to 10 or 15 mm in a two-step approach will reduce the number tested with QFT at the cost of number identified with latent infection (Table 6).

Discussion

The performance of new and specific blood tests for detecting latent infection has now been studied in several contexts, including contact tracing and screening of defined risk groups (27, 28, 33-36). We have in this work recruited a large number of newly arrived asylum seekers to compare the performance of TST and QFT. We have used the results to evaluate the role of QFT in screening immigrants for latent infection on arrival in a low-prevalence country. The study is part of a more extensive research protocol, where longitudinal data will be observed.

The prevalence of latent infection among the asylum seekers was high, both defined by TST (50%) and QFT (29%), as expected in a population predominantly arriving from high prevalence countries. Our findings are similar with WHO estimates based partly on TST studies (3). Most asylum seekers from Asia came from relatively low prevalence countries, while many Africans came from Somalia where the prevalence is probably very high.

Agreement between QFT and TST

Kappa statistics measures agreement beyond the level of agreement expected by chance alone. The observed agreement is the proportion of samples where the two tests are concordant in their results. Kappa has a maximum value of one where there is perfect agreement and a kappa value of zero indicates agreement no better than chance alone (50). As a guideline Altman has described the strength of agreement as poor for kappa-values of < 0.20 , fair if Kappa is $0.21 - 0.40$, moderate if Kappa is $0.41 - 0.60$, good if Kappa is $0.61 - 0.80$ and as very good agreement if Kappa is $0.81 - 1.00$ (51). Consistent with other studies, we found a moderate agreement between the results of QFT and TST for most groups, although differences in design and populations make comparison between such studies difficult (27, 34, 35, 39, 52). The moderate agreement was as expected as we know the QFT has improved specificity compared to TST.

Importantly, we observed that the proportion of individuals with positive QFT increased with increasing TST induration. This is consistent with the expected association between the increase in TST indurations and the proportion of tuberculin reactions caused by *M. tuberculosis* (24). A TST reaction of ≥ 15 mm has been interpreted as high likelihood of true tuberculosis infection (23, 25).

Discordant results in our study were found in the range of 21 - 29% depending on the TST-cut-off value, but mainly as positive TST and negative QFT in the range of 6-9 mm (72%) and 10-14 mm (49%). The poor specificity of TST in these intervals is a major challenge as most positive TST results (69%) occurred within this range as well as 48% of the positive QFT-results. False positive TST indurations caused by BCG or non-tuberculosis mycobacteria are expected to be moderate and rarely exceeding 15 mm (23, 25, 28, 53). Boosted TST reactions or differences in the nature of the immune-response measured by the two tests might also explain discordant results (33, 54). In this study the blood sample was drawn when the TST was read. A potential influence of PPD exposure on a QFT test has been investigated. Having a QFT test after TST is advised to be a reliable approach (55). This is also in agreement with the European Consensus Report, where it is advised that IGRA should follow a positive TST (56).

Possible explanations for discordant results

Effect of previous BCG-vaccination

A meta analysis reported increased likelihood of a positive TST in BCG-vaccinated persons within the first 15 years after vaccination (57). We found no significant effect of previous BCG-vaccination on QFT or on TST-results in multivariate regression analyses, regardless of TST cut-off 15, 10 or 6 mm. The limited effect of BCG observed in our study might be explained by the fact that vaccination is most often offered to newborns and all our participants were older than 18 years. In addition, scars might be underreported either by being missed or being misinterpreted by the observer, or vaccinated persons might not have developed a scar. Non-vaccinated persons in this study were to a large extent from Somalia, a population where the prevalence of latent infection is high. This might explain the better agreement between the tests for non-vaccinated compared to vaccinated participants. As revaccination policies have been common in some European countries (including Russia) the stronger effect of BCG-vaccination might explain the lower agreement found among European participants.

Differences in immunological responses measured by the two tests

Tuberculin induces a delayed-type hypersensitivity reaction reflecting a memory response, while the blood tests detect cellular INF- γ release reflecting an effector response, to an ongoing infection. Due to these differences in immunological responses it is suggested that IFN- γ release assays detect more than TST those with recent and persistent infection (33, 39). This can partly explain discordance and might have important clinical and programmatic implications as recent infection is more likely to progress to active disease and preventive treatment is therefore more indicated. There is some ongoing research to determine whether the level of IFN- γ response to the specific *M. tuberculosis* antigens may be meaningful for predicting the outcome of latent infection (58).

Differences in sensitivity between the tests

Among participants with a strongly positive TST (≥ 15 mm) we found that 26% (37 out of 141) had a negative QFT test. High proportions of discrepant results in this group has also been observed in other studies (47, 59, 60). Among immune-competent persons the sensitivity of tuberculin is regarded as very high, while sensitivity of QFT for detection of latent infection has been variable (75-97%) depending on the study population and design (37-39). Among patients with tuberculosis disease confirmed by culture sensitivity measurements for the two tests vary in different studies, but a recent study showed similar sensitivity between the tests (28, 30, 38). The fact that we found one QFT negative individual among four participants with confirmed active disease demonstrates the need for careful evaluation of test results. The main purpose of TST and QFT used in screening is to identify persons with latent infection eligible for

preventive treatment. Screening of symptoms and chest X-ray would have identified all five asylum seekers with active tuberculosis in the study. Among participants with negative TST, we found that 7% (32 out of 452) had a positive QFT. This might reflect false negative TST results as IGRA has shown to have higher sensitivity than TST among immune-suppressed individuals (61, 62).

Due to the lack of a positive control for a functional immune response (mitogen stimulation) in the QFT assay available when the study was conducted, the general immune status of the participants were not known. This is a limitation of the study and may in cases of severe immune-suppression have lead to false negative results with respect to both QFT and TST. Due to ethical considerations it was not acceptable to test asylum seekers for hiv-infection on the day of arrival. However, recent information on the performance of the QFT in immune-suppressed individuals demonstrates that only severe immune-suppression with low CD4 counts may lead to indeterminate results or potentially false negative results in case of no positive mitogen control (40-43). Based on surveillance of hiv-infection in Norway we have no reason to believe that severe immune-suppression was affecting more than very few cases, if any at all, among our participants. We do not think the lack of a positive mitogen control in this study represents any major problem for the interpretation of the results. As the present QFT assay now contains a positive mitogen control it is possible to avoid potential false negative results.

Predictors of positive tests

Multivariate analysis is a technique to predict the outcome of a single dependent variable from the values of several other explanatory (predictor) variables. Multivariate regression analysis is used to identify variables that for example can predict an outcome of binary variables, such as being QFT-positive or negative. In a logistic regression, a logit transformation is normally applied to the model. Four different logistic regression analyses were performed in this study. Odds ratios calculated from the estimated coefficients in the final models were used to measure the strength of association. Odds ratio is the ratio of the odds of occurrence of an event given a certain value of the covariate and the corresponding odds for another value of the covariate.

We wanted to investigate significant predictors for positive TST (different cut-offs) and for a positive QFT and also whether predictors of a positive TST and QFT were similar. As both TST indurations ≥ 15 mm and positive QFT most likely reflect true tuberculosis infection it was interesting to assess differences and similarities among them.

The multivariate analyses showed similar significant risk factors for positive QFT and TST >15 mm. Country of origin and increase in age were both significantly associated with both positive QFT and TST ≥ 15 mm, reflecting accumulated exposure time and background prevalence in country of origin. Exposure to an infectious case of tuberculosis was, as found in other studies, significantly related to QFT but not to TST

(27, 33, 34, 43, 63, 64). Previous history of tuberculosis was not a significant predictor for either test as it correlated with exposure.

Why female gender was a predictor for positive TST ≥ 10 mm, but not for a positive QFT is unclear. There is no simple explanation and it is not much assessed in other studies. Age, origin and previous history of BCG-vaccination are controlled for in the regression analyses. Women could potentially be more exposed to non-tuberculosis mycobacteria than men through every day chores and work, but this information is not available in the study. Another factor could be multiple BCG-vaccinations that would affect TST, but not QFT, but this does not fit well with the origin of the women and current vaccination practices world wide. Assessment of gender differences needs further studies.

Implications for screening in Norway

The ideal potential test would identify LTBI with high accuracy and have the ability to predict progression to tuberculosis disease. Ideally a new test should be validated against an established gold standard in an appropriate spectrum of persons. Although the tuberculin is the test currently used for detection of latent infection it cannot be considered a gold standard because of the number of false positive and false negative reactions, and the variability of the interpretation. Both positive QFT and TST ≥ 15 mm (independent of each other) have been suggested to accurately identify latent infection and also predict progression to active disease. We have, for the purpose of assessing the use of QFT in screening, defined all with a positive QFT and all with TST ≥ 15 mm as having latent infection and therefore the target group for further follow up. Based on this definition, a more specific test than TST for diagnosis of latent infection is clearly needed as TST with cut-off at 6 mm or 10 mm results in referral of 42% or 27% false positive cases respectively, while a cut-off of 15 mm will detect less than half of the actual cases. As it is neither feasible nor cost effective to screen all asylum seekers with both tests, the options are to replace TST with QFT (one step approach) or to use QFT to confirm a positive TST (two step approach).

A one step approach with only QFT has logistic advantages as only one visit to health care facilities is required and referral based on a false positive TST is avoided. The strength of this approach would be the inclusion of the TST negative among the QFT positive participants (7%), a presumable high-risk group for active disease. These cases would be lost in a two step approach.

The two step approach allows for referral procedures based on both tests and it is recommended in a European consensus report from 2006 (56). This strategy follows normal procedures for screening with a first test with high sensitivity followed by a second test with high specificity. The two-step approach identifies and refers all TST positive cases (≥ 6 mm) with a positive QFT-result and also the 12% with TST ≥ 15 mm and a negative QFT. These cases would be lost in a one step approach (Table 6).

If ≥ 6 mm is chosen as cut-off for TST the three approaches would identify a similar proportion (88-89%) of persons defined as having latent infection, even though it would not be the same persons referred. By implementing QFT the number referred for preventive treatment would be reduced by 42% , independent of whether the one- or two step approach is chosen. Sensitivity and specificity are interdependent, and a gain in sensitivity is offset by a loss of specificity and vice versa. Adjusting TST cut-off value to 10 or 15 mm would reduce the number referred to 25% and 15%, respectively, but cases with latent infection lost would consequently increase to 25% and 53%. Whether a one step approach with only QFT is feasible depends also on laboratory capacity. Cost implications of the different screening approaches need further study. Based on the present study the Norwegian Institute of Public Health and its Tuberculosis Advisory Committee have recommended to the Ministry of Health the use of QFT in a two step approach for screening of immigrants in Norway. An article from the study has been accepted for publication in BMC Infectious Diseases.

Strengths and weaknesses of the study

The purpose of this discussion is to summarize the methodological advantages and limitations of the study and to assess the extent to which such limitations may have affected the results.

The sample size of the study was large and all participants were tested with both tests, compared to most other studies where both factors often are limitations. The sample size was sufficiently large to answer the research questions in the aim of the study and contributed important information on vulnerable subgroups.

Bias

The overall goal of an epidemiological study is to estimate the value of the parameter which is the object of measurement with as little error as possible. Sources of error may be classified as random or systematic. Random error affects the precision of the study, while the presence of systematic errors including selection bias, information bias and confounding influences the validity of the study (65).

Random error

Random error has random size and direction, cannot be predicted and occurs by chance. The primary way of reducing random error and thereby improving precision is to enlarge the size of the study. The one occasion that caused exclusion of 44 tests due to technical mistakes in the laboratory was unfortunate and represents a random error in

this study. It happened once and there is no reason to believe these 44 participants were significantly different from the study group as a whole. The same applies to the three tests discarded due to routine error during sampling. These 47 exclusions are not expected to significantly affect the precision of this study, due to the large sample size, as they represent less than 5% of the enrolled participants.

Systematic error

Systematic error (bias) has constant size and direction and is repeated throughout the study. A systematic error will systematically over- or understate the results and may be related to how the subjects were selected, how the study variables were measured or some confounding factor that is not completely controlled for.

Selection bias

Selection bias results from systematic differences between characteristics of the study population and the target population. As all asylum seekers arriving in the study period were invited to participate, a potential bias would refer to systematic differences between the group who consented to participation and those who did not, a non-response bias. Only 36% of the eligible asylum seekers agreed to participate. As the TST result was known to the participants when the informed consent was signed we were worried that participants with negative or small TST indurations would be more likely to reject participation. Comparison of the study group with the control group of non-participants showed no significant differences on important variables like the size of the TST induration, age or previous BCG-vaccination. Thus, the study group seems fairly representative for the asylum seeker population arriving in the study period. As referral is based on the TST-result, representativity on this variable is of most importance.

The fact that fewer European asylum seekers participated in the study, could potentially influence the overall agreement between the tests found in the study. Revaccination policies have been common in some European countries (including Russia) and the influence of BCG vaccination on the result of tuberculin skin testing is clearly related to the time elapsed since vaccination (1). The stronger effect of BCG-vaccination expected among European asylum seekers could potentially have increased the number with discordant results if the study group was representative for all arrivals. As a TST > 15 mm is expected to reflect tuberculosis infection and the QFT is not affected by previous vaccination this bias is not expected to affect the conclusions of our study. Stronger stigma related to tuberculosis and poorer language skills can probably explain why female asylum seekers were less likely to participate in the study. The study identified female gender as a predictor of positive TST results while gender was not a predictor for a positive QFT result as discussed on page 27. The selection bias could therefore influence the agreement between the tests found in the study, but is less likely to affect the outcome of the screening model.

Eighty-eight participants enrolled in the study were later excluded from analysis for different reasons (Figure 2). Exclusion of the 35 cases without a confirmatory positive QFT is of most concern as this can represent a systematic error in the study and might cause underestimation of both positive and negative results. The cases (34/35) had both one positive and one negative result and many had results clustering at or relatively near the cut off value (0,35 U/ml) for the QFT test. It is not surprising that samples with this cytokine level comes out as positive (just above cut-off) in one analysis and as negative (just below cut-off) in a second analysis, as with all cut-off based analyses. Compared to the large number of samples analyzed in this study this has to be considered as an acceptable problem with such tests and also the 35 tests do not represent more than approximately 3% of all tests in the study. We do not think the exclusions have significant impact on the conclusions of our study. We have run the multivariate analysis with QFT as outcome variable with the 35 cases first included as positive and then as negative cases and we found this to have no significant impact on predictors of positive tests and the exclusions are not affecting the discussion or conclusion for this part. We also modified Table 6 with the different screening options and included the 35 cases as both positive and negative cases. There is a difference in numbers, but as we see it the relative difference between the screening options are the same and the exclusions will not affect the conclusions of our study. In another study we have performed we had the opportunity to collect new blood samples from eight participants with a negative confirmatory test. All eight now had valid test results; six became negative and two positive. It is therefore hard to predict how many of the 35 participants most correctly should be included as negative and how many as positive cases. We found it incorrect to include the 35 participants in our study since this would potentially over- or underestimate the number of QFT positives respectively. In a normal screening setting, this will not represent any significant problem as it will be possible to analyse on a new sample that can lead to a conclusive result.

Information bias

TST

There are well known significant obstacles to reliability of TST, such as boosting and reader bias and as seen in many TST studies we identified a clear digit preference to cut-off points for TST (Figure 3b). Ideally double reading should have been performed throughout the study. This was not feasible due to the routine setting the study was conducted in. Systematic differences in reading between the nurses cannot be ruled out, but they had recently been through training where double reading was performed and major difference was not detected.

QFT

As with all tests a cut-off is defined to balance sensitivity versus specificity of a test. This study was not designed to assess appropriate cut-off values for QFT and like in most studies the manufacturer defined cut-off was used. It is still important to acknowledge that while results of QFT are treated as dichotomous outcomes, the level of IFN- γ is also a continuous measure. The fact that only positive results were re-

analyzed is of some concern as we don't know if re-analysis of negative results would have lead to more non-conclusive results, even though this is less likely. Negative tests were not re-analyzed as this was not feasible due to the work load and discordant results were less likely to occur.

There is always a possibility that information bias might occur when people are asked to recall past events or happenings. The variable "known exposure" was based on information from the participating asylum seekers. Some might not know of any episode of exposure as tuberculosis is an airborne disease; they might define exposure differently or might not want to disclose any risk for an infectious disease as they are new in a country. The validity of this information might be limited. However we found QFT to be associated with exposure despite the few cases and all cases with disease reported known exposure.

Confounding

Confounding can be considered as mixing of effects. The estimate of the effect of one factor is distorted because it is mixed together with the effect of another factor. A confounder must be associated with both the outcome and with a factor under investigation. The failure to control for a confounding can lead to over- or underestimation of an effect, depending on the direction of the distortion. Confounding is often detected at an early stage of statistical analyses, although possible confounders may also been known before the study starts. Variables such as sex, age, origin and previous BCG-vaccination may have a confounding effect, but they are controlled for in the regression analyses.

There may be confounders which we have not adjusted for in the analyses, like the general immune status of the participants, hiv-status most importantly, multiple scars from previous BCG-vaccinations and different levels of exposure to non-tuberculosis mycobacteria. This is a limitation of the study. These factors might influence the outcome of the study and has been discussed in the interpretation of the results. Information on multiple BCG-scars would be available and possibly could have refined interpretation and data analysis. Ethical considerations inherent in the study design limited access to the more important information on general immune status.

Assessment of external validity

External validity reflects to what extent the conclusions in a study can be generalized to a broader population of interest, for example asylum seekers arriving in Norway in the future. Internal validity is a prerequisite for, but does not guarantee external validity. As the asylum seeker population will change over time reflecting the current political and disaster situation in the world, the external validity can always be questioned. On the other hand, the study population should be representative for a wider asylum seeker population since they represent all arrivals and the sample size in the study is large. The

major groups arriving as asylum seekers in Norway are fairly stable over time, even with some areas being overrepresented from time to time due to sudden global incidents. The number in need for further medical evaluation might differ somewhat in different time periods, but implications of the suggested screening approach will still give a reasonable estimate of the proportion in need of further follow-up.

Conclusions

The prevalence of latent infection among asylum seekers was high, both defined by TST (50%) and QFT (29%), and with an expected discordance between the two test methods. There is an urgent need for a more specific tool than TST for diagnosis of latent infection in routine screening, and the increased specificity of QFT is in this context a very important improvement. By implementing QFT in routine screening, the number of persons referred for preventive treatment would be reduced by 42% independent of whether the one- or two-step approach is implemented, compared to referral based on TST (≥ 6 mm) alone. All three screening approaches would identify a similar proportion (88 - 89%) of those defined as having latent infection in this study, even though it would not be the same persons referred. Based only on QFT and TST results a small group will be missed for further follow up, unless all asylum seekers are screened with both tests. Persons who are TST negative and QFT positive, and therefore lost in a two step approach, could be identified by screening of symptoms, chest X-ray or other diagnostics. Hiv-testing is also offered to asylum seekers at a later time. As only 0.5 % of the study group had active tuberculosis disease on arrival, identification of persons with latent infection for referral and assessment for preventive treatment remains a key objective of the screening on entry.

We recommend for future studies to focus on the predictive value of IGRA for the development of disease in large prospective studies and quantify the risk of progression to disease in TST negative, but QFT positive adults as well as TST ≥ 15 mm and QFT negative adults. Cost effectiveness for different screening approaches should also be better informed.

Acknowledgements

This is a large study with the involvement of many professionals and I greatly want to thank all my colleagues who have participated in this study.

First Baerum Municipal Health authorities and all staff at Tanum reception centre who have contributed with their expert assistance with tuberculin skin test administration and reading. Anette Skistad Jeppesen has coordinated the study at the reception centre.

Ingunn Harstad from the Norwegian University of Science and Technology, Faculty of medicine, has contributed with interpretation of chest x-ray results and in the writing process.

Fredrik Oftung, Gro Ellen Korsvold and Turid Mannsåker, all from Department of Bacteriology and Immunology at the Norwegian Institute of Public Health have been responsible for the laboratory services, including analysis of QFT, interpretation of the results and valuable contribution in the writing process.

Max Petzold from the Nordic School of Public Health has been a tutor for the Master Thesis and has contributed substantially to the public health approach and presentation of statistical and descriptive data in the study.

Administrative staff at the Norwegian Institute of Public Health has contributed substantially to the logistics of the study. Einar Heldal, Berit Tafjord Heier and Preben Aavitsland from Department of Infectious Disease Epidemiology at the Norwegian Institute of Public Health have supervised the study. Berit Tafjord Heier on statistical analyses, Preben Aavitsland with valuable input in the writing process and Einar Heldal as the expert in the field of tuberculosis.

Financial support: The Norwegian Health Association funded the study and the Norwegian Institute of Public Health contributed with human resources.

References

1. Rieder HR. Epidemiological Basis of Tuberculosis Control. Paris: International Union against Tuberculosis and Lung Disease; 1995.
2. Global tuberculosis control: surveillance, planning, financing : WHO report 2007. Geneva: World Health Organization; 2007.
3. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA 1999;282:677-86.
4. Romanus V, Tala E, Blondal T, Heldal E, Poulsen S. [Tuberculosis control in Scandinavia]. Nord Med 1995;110:45-7.
5. EuroTB and the national coordinators for tuberculosis surveillance in the WHO European Region. Surveillance of tuberculosis in Europe. Report on tuberculosis cases notified in 2005. Saint Maurice, France: Insitut de Veille Sanitaire; 2007.
6. Winje BA, Mannsåker T, Heldal E, Dahle UR. Tuberkulose i Norge 2006 (Norwegian only). MSIS-rapport 2007;35
7. Winje BA, Mannsåker T, Blystad H, Dahle U. Tuberkuløs sykdom meldt i Norge i 2003 (Norwegian only). MSIS-rapport 2004;32
8. Winje BA, Mannsåker T, Blystad H, Heldal E, Dahle UR. Tuberkuløs sykdom meldt i Norge i 2004 (Norwegian only). MSIS-rapport 2005;26
9. Winje BA, Mannsåker T, Heldal E, Brantsaeter AB, Dahle UR. Tuberkulose i Norge i 2005 (Norwegian only). MSIS-rapport 2006;33
10. Dahle UR, Sandven P, Heldal E, Caugant DA. Continued low rates of transmission of Mycobacterium tuberculosis in Norway. J Clin Microbiol 2003;41:2968-73.
11. Dahle UR, Eldholm V, Winje BA, Mannsaker T, Heldal E. Impact of immigration on the molecular epidemiology of Mycobacterium tuberculosis in a low-incidence country. Am J Respir Crit Care Med 2007;176:930-5.
12. Farah MG, Tverdal A, Selmer R, Heldal E, Bjune G. Tuberculosis in Norway by country of birth, 1986-1999. Int J Tuberc Lung Dis 2003;7:232-5.

13. Broekmans JF, Migliori GB, Rieder HL, Lees J, Ruutu P, Loddenkemper R, et al. European framework for tuberculosis control and elimination in countries with a low incidence. Recommendations of the World Health Organization (WHO), International Union Against Tuberculosis and Lung Disease (IUATLD) and Royal Netherlands Tuberculosis Association (KNCV) Working Group. *Eur Respir J* 2002;19:765-75.
14. Last JM. Last JM, ed. *A Dictionary of Epidemiology*. 4 ed. Oxford University Press; 2001.
15. Folkehelseinstituttet. Forebygging og kontroll av tuberkulose: en veileder (Guidelines for prevention and control of tuberculosis). 7 ed. Oslo: Folkehelseinstituttet; 2002.
16. Helsedepartementet. Forskrift om tuberkulosekontroll: forskrift av 21. juni 2002 nr. 567. [Oslo]: Departementet; 2002.
17. Targeted tuberculin testing and treatment of latent tuberculosis infection. American Thoracic Society. *MMWR Recomm Rep* 2000;49:1-51.
18. Purified protein derivative (PPD)-tuberculin anergy and HIV infection: guidelines for anergy testing and management of anergic persons at risk of tuberculosis. *MMWR Recomm Rep* 1991;40:27-32.
19. Caiaffa WT, Graham NM, Galai N, Rizzo RT, Nelson KE, Vlahov D. Instability of delayed-type hypersensitivity skin test anergy in human immunodeficiency virus infection. *Arch Intern Med* 1995;155:2111-7.
20. Graham NM, Nelson KE, Solomon L, Bonds M, Rizzo RT, Scavotto J, et al. Prevalence of tuberculin positivity and skin test anergy in HIV-1-seropositive and -seronegative intravenous drug users. *JAMA* 1992;267:369-73.
21. Huebner RE, Schein MF, Bass JB, Jr. The tuberculin skin test. *Clin Infect Dis* 1993;17:968-75.
22. Rose DN, Schechter CB, Adler JJ. Interpretation of the tuberculin skin test. *J Gen Intern Med* 1995;10:635-42.
23. Bahrmand AR, Madani H, Samar G, Khalilzadeh L, Bakayev VV, Yaghli M, et al. Detection and identification of non-tuberculous mycobacterial infections in 6,472 tuberculosis suspected patients. *Scand J Infect Dis* 1996;28:275-8.
24. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 1974;99:131-8.
25. von Reyn CF, Horsburgh CR, Olivier KN, Barnes PF, Waddell R, Warren C, et al. Skin test reactions to *Mycobacterium tuberculosis* purified protein derivative

and *Mycobacterium avium* sensitin among health care workers and medical students in the United States. *Int J Tuberc Lung Dis* 2001;5:1122-8.

26. Andersen P, Munk ME, Pollock JM, Doherty TM. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000;356:1099-104.
27. Pai M, Riley LW, Colford JM, Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis* 2004;4:761-76.
28. Kang YA, Lee HW, Yoon HI, Cho B, Han SK, Shim YS, et al. Discrepancy between the tuberculin skin test and the whole-blood interferon gamma assay for the diagnosis of latent tuberculosis infection in an intermediate tuberculosis-burden country. *JAMA* 2005;293:2756-61.
29. Mazurek GH, Weis SE, Moonan PK, Daley CL, Bernardo J, Lardizabal AA, et al. Prospective comparison of the tuberculin skin test and 2 whole-blood interferon-gamma release assays in persons with suspected tuberculosis. *Clin Infect Dis* 2007;45:837-45.
30. Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K, et al. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. *Am J Respir Crit Care Med* 2004;170:59-64.
31. Pai M, McCulloch M, Enanoria W, Colford JM, Jr. Systematic reviews of diagnostic test evaluations: What's behind the scenes? *ACP J Club* 2004;141:A11-A13.
32. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann Intern Med* 2007;146:340-54.
33. Arend SM, Thijsen SF, Leyten EM, Bouwman JJ, Franken WP, Koster BF, et al. Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. *Am J Respir Crit Care Med* 2007;175:618-27.
34. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 2003;361:1168-73.
35. Lalvani A, Pathan AA, Durkan H, Wilkinson KA, Whelan A, Deeks JJ, et al. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Lancet* 2001;357:2017-21.
36. Richeldi L, Ewer K, Losi M, Bergamini BM, Roversi P, Deeks J, et al. T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. *Am J Respir Crit Care Med* 2004;170:288-95.

37. Lalvani A, Richeldi L, Kunst H. Interferon gamma assays for tuberculosis. *Lancet Infect Dis* 2005;5:322-4.
38. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm Rep* 2005;54:49-55.
39. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: part I. Latent tuberculosis. *Expert Rev Mol Diagn* 2006;6:413-22.
40. Brock I, Ruhwald M, Lundgren B, Westh H, Mathiesen LR, Ravn P. Latent tuberculosis in HIV positive, diagnosed by the *M. tuberculosis* specific interferon-gamma test. *Respir Res* 2006;7:56.
41. Jones S, de Gijzel D, Wallach FR, Gurtman AC, Shi Q, Sacks H. Utility of QuantiFERON-TB Gold in-tube testing for latent TB infection in HIV-infected individuals. *Int J Tuberc Lung Dis* 2007;11:1190-5.
42. Kobashi Y, Mouri K, Obase Y, Fukuda M, Miyashita N, Oka M. Clinical evaluation of QuantiFERON TB-2G test for immunocompromised patients. *Eur Respir J* 2007;30:945-50.
43. Luetkemeyer AF, Charlebois ED, Flores LL, Bangsberg DR, Deeks SG, Martin JN, et al. Comparison of an interferon-gamma release assay with tuberculin skin testing in HIV-infected individuals. *Am J Respir Crit Care Med* 2007;175:737-42.
44. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann Intern Med* 2007;146:340-54.
45. Diel R, Loddenkemper R, Meywald-Walter K, Niemann S, Nienhaus A. Predictive Value of a Whole-blood IFN- γ Assay for the Development of Active TB Disease. *Am J Respir Crit Care Med*, in press 2008;
46. Lalvani A, Nagvenkar P, Udwadia Z, Pathan AA, Wilkinson KA, Shastri JS, et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. *J Infect Dis* 2001;183:469-77.
47. Mahomed H, Hughes EJ, Hawkrigde T, Minnies D, Simon E, Little F, et al. Comparison of mantoux skin test with three generations of a whole blood IFN-gamma assay for tuberculosis infection. *Int J Tuberc Lung Dis* 2006;10:310-6.
48. Carvalho AC, Pezzoli MC, El Hamad I, Arce P, Bigoni S, Scarcella C, et al. QuantiFERON((R))-TB Gold test in the identification of latent tuberculosis infection in immigrants. *J Infect* 2007;55:164-8.

49. Utlendingsdirektoratet. Tall og fakta 2006 [http://www.udi.no/upload/Presse/TallOgFakta/2006/Tall_Fakta.pdf]. [updated 2008 Mar 11; cited . Available from: <http://www.udi.no/templates/Statistikk.aspx?id=8594> .
50. Altman DG, Machin D, Bryant T N, Gardner M J, eds. Statistics with confidence. Confidence intervals and statistical guidelines. 2. ed. BMJ Books; 2000.
51. Altman DG. Practical statistics for medical reserach. First edition ed. Washington DC, USA: Chapman&Hall/CRC; 1991.
52. Shams H, Weis SE, Klucar P, Lalvani A, Moonan PK, Pogoda JM, et al. Enzyme-linked immunospot and tuberculin skin testing to detect latent tuberculosis infection. *Am J Respir Crit Care Med* 2005;172:1161-8.
53. Berkel GM, Cobelens FG, de Vries G, Draayer-Jansen IW, Borgdorff MW. Tuberculin skin test: estimation of positive and negative predictive values from routine data. *Int J Tuberc Lung Dis* 2005;9:310-6.
54. Nguyen M, Perry S, Parsonnet J. QuantiFERON-TB predicts tuberculin skin test boosting in U.S. foreign-born. *Int J Tuberc Lung Dis* 2005;9:985-91.
55. Leyten EM, Prins C, Bossink AW, Thijsen S, Ottenhoff TH, van Dissel JT, et al. Effect of tuberculin skin testing on a Mycobacterium tuberculosis-specific IFN- γ assay. *Eur Respir J* 2007;29:1212-6.
56. Drobniewski F, Cobelens F, Zellweger J-P. Use of Gamma-interferon assays in low- and medium-prevalence countries in Europe: a consensus statement of a Wolfheze Workshop organised by KNCV/EuroTB, Vilnius Sept 2006. *Eurosurveillance weekly* 2007;12
57. Wang L, Turner MO, Elwood RK, Schulzer M, FitzGerald JM. A meta-analysis of the effect of Bacille Calmette Guerin vaccination on tuberculin skin test measurements. *Thorax* 2002;57:804-9.
58. Leyten EM, Arend SM, Prins C, Cobelens FG, Ottenhoff TH, van Dissel JT. Discrepancy between Mycobacterium tuberculosis-specific gamma interferon release assays using short and prolonged in vitro incubation. *Clin Vaccine Immunol* 2007;14:880-5.
59. Hill PC, Brookes RH, Fox A, Fielding K, Jeffries DJ, Jackson-Sillah D, et al. Large-scale evaluation of enzyme-linked immunospot assay and skin test for diagnosis of Mycobacterium tuberculosis infection against a gradient of exposure in The Gambia. *Clin Infect Dis* 2004;38:966-73.
60. Pai M, Gokhale K, Joshi R, Dogra S, Kalantri S, Mendiratta DK, et al. Mycobacterium tuberculosis infection in health care workers in rural India: comparison of a whole-blood interferon gamma assay with tuberculin skin testing. *JAMA* 2005;293:2746-55.

61. Chapman AL, Munkanta M, Wilkinson KA, Pathan AA, Ewer K, Ayles H, et al. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. *AIDS* 2002;16:2285-93.
62. Mazurek GH, et al. Prospective Comparison of the Tuberculin Skin Test and 2 Whole-Blood Interferon- γ Release Assays in Persons with Suspected Tuberculosis. *Clin Infect Dis* 2007;837-45.
63. Brock I, Weldingh K, Lillebaek T, Follmann F, Andersen P. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. *Am J Respir Crit Care Med* 2004;170:65-9.
64. Diel R, Nienhaus A, Lange C, Meywald-Walter K, Forssbohm M, Schaberg T. Tuberculosis contact investigation with a new, specific blood test in a low-incidence population containing a high proportion of BCG-vaccinated persons. *Respir Res* 2006;7:77.
65. Rothman KJ. *Epidemiology, an Introduction*. New York: Oxford University Press, Inc; 2002.

Attachments

Attachment 1

Information letter from the Norwegian Institute of Public Health

No. 1 Tuberculosis • English • Last updated September 2005

www.fhi.no/publ/infoletter



It is important to treat tuberculosis

What is tuberculosis? Tuberculosis is a serious infectious disease caused by the bacterium *M. tuberculosis*. The disease may affect all organs of the body, but it most frequently affects the lungs. Tuberculosis is curable with early detection and correct treatment. Tuberculosis treatment is free of charge in Norway.

How is tuberculosis spread? Tuberculosis is spread through the air. Only persons with tuberculosis in the lungs can infect others. Some have tuberculosis in their lungs without feeling ill. Even so, they can infect others.

When a person with tuberculosis in the lungs speaks, coughs or sneezes, the bacteria are spread in the air. Other persons can then inhale the bacteria. Tuberculosis is therefore mainly spread among persons living closely together.

Development of illness Disease can develop many years after transmission occurred. Not everyone infected develops the disease.

Tuberculosis symptoms occur from the organs of the body that are affected. Common symptoms of tuberculosis in the lungs are long term cough, fever, night sweat and weight loss.

Treatment of tuberculosis Tuberculosis is usually treated with tablets for six months. To be cured it is vital that the treatment is completed in the correct manner. All tuberculosis treatment in Norway is therefore given as directly observed treatment. This means that health personnel observe that the patient takes his/her medication as the doctor has prescribed.

Some persons may be offered treatment of infection before they develop the disease. This is called preventive treatment. This is especially important for young children who develop serious forms of tuberculosis more easily than adults.

Mandatory tuberculosis examination Tuberculosis examination is mandatory for some groups. This includes, among others, persons from countries where tuberculosis is common who will be spending more than three months in Norway. The examination consists of a skin test, possibly a blood sample and a chest x-ray for persons over 15 years of age.

The aim of the examination is to:

- detect the disease as early as possible in order to offer treatment and prevent further spread
- detect persons who are infected with tuberculosis but not ill in order to consider preventive treatment
- offer vaccination (BCG) to persons who are not infected and who are not vaccinated

It is also important that persons without legal residency in Norway are examined for tuberculosis. Test results that indicate tuberculosis infection will not have negative consequences for work or resident permit applications.

Disease can develop many years after transmission occurred. Transmission of tuberculosis can also occur during longer visits to countries where tuberculosis is common. It is important to contact a doctor as soon as possible if symptoms of tuberculosis are observed.

If you would like more information about tuberculosis or wish to be examined, please contact your family doctor/regular general practitioner, your municipal medical officer or the nearest health centre. Personnel at the asylum centres can also offer advice and guidance as to where you can get medical help.



Request to participate in a study

The result of your tuberculin test has just been read. The tuberculin test is used to determine if you are infected with tuberculosis. If there is a swelling around the injection site, it is measured, and the size determines if it is considered positive or negative. The tuberculin test can also be positive for other reasons than infection with tuberculosis. The result may therefore be hard to interpret, and it may be difficult for the doctor to determine correct follow-up. A new method for diagnosing tuberculosis infection with a blood sample has been developed. This method will be more exact in determining if you are infected with tuberculosis or not.

The Norwegian Health Association and the Norwegian Institute of Public Health are involved in a study to investigate if the new blood sample method will improve the detection of tuberculosis infection among asylum-seekers in Norway.

The name of the study is:

QuantiFERON® TB Gold testing for tuberculosis infection among asylum seekers upon arrival in Norway

This is a request for you to participate in an evaluation of the new blood sample test regardless of the result of your tuberculin test. The project will continue through 2006.

Participating in the study means that:

- We take a blood sample from your arm. This will be done while you are here at Tanum asylum centre.
- The blood sample is analyzed with the new method.
- You and your doctor will be informed of the result of the blood sample. This will give you and your doctor a more certain and better answer to the question of whether or not you are infected with tuberculosis.

It is important to know that:

- Participating in the study will not affect your application for asylum in Norway.
- Your blood sample will not be examined for other diseases
- The samples will be stored with a code, and only the contact person at Tanum asylum centre has access to the code. In the study we will only receive the result of the blood sample, the tuberculin test and the chest x-ray and a few other data. Your name will not be revealed to us.

- Your blood sample will be stored in the laboratory of the Norwegian Institute of Public Health for the duration of the study. The Director of the Division for Infectious Disease Control at the Norwegian Institute of Public Health, Hanne Nøkleby, is responsible for the laboratory. When the study is over, and no later than 01.01.2007, the blood sample will be destroyed and discarded.

Remember:

Participation in this study is voluntary and you can ask for time to think it over. You may withdraw from the study at any time and we will not use your results in the project. You will not be asked to explain why you do not wish to participate or why you withdraw. You have the right to ask questions about the study and the right to demand that the blood sample is destroyed and discarded and that the information collected concerning your health is deleted. This must happen before the data are included in scientific reports or publications.

If you require any other information about the study please contact Brita Winje at the Norwegian Institute of Public Health, PO. Box 4404, Nydalen, 0403 Oslo or telephone 22 04 25 94. The contact person at Tanum asylum centre is Anette Skistad Jeppesen. You will be in direct contact with her.

Declaration of consent

I have received written and oral information about the study and agree to participate.

Date

Signature

