ORIGINAL ARTICLE

Prognostic value of interferon-γ release assays, a population-based study from a TB low-incidence country

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ABSTRACT

Background The ability of interferon-γ release assays to predict the development of TB has been investigated in many studies, but few cases develop TB during follow-up limiting the generalisation of results.

Methods We assessed QuantiFERON-TB Gold In-Tube test (QFT) results from 15 980 Danish individuals and data on all TB cases in Denmark from 2005 to 2012 and determined the predictive value of the QFT for coprevalent TB (0–90 days after testing) and incident TB (>90 days).

Results Coprevalent TB was diagnosed in 10.7% (183/1703) and 0.3% (38/13 463) cases with a positive and negative QFT, respectively. For the QFT-positive cases, coprevalent TB was more frequent among persons <35 years compared with those >35 years (19.3% vs 7.2%, p<0.001). The cohort was followed-up for 52 807 person-years, median follow-up time was 3.36 years. For incident TB, the positive and negative predictive values (PPV and NPV) were 1.32% and 99.85%, respectively. Incidence rates (IR) for incident TB among QFT-positives and QFT-negatives were 383 per 10^5 and 45 per 10^5 person-years, respectively. Among cases with a positive QFT, IR for incident TB was associated with time interval since QFT (<2 years, p<0.001), but not with age (<35 years, p=0.087).

Conclusions We confirmed a high NPV of the QFT and found positive QFT associated with a higher risk of subsequent incident TB. Overall, the PPV for incident cases was 1.32%, and development of incident TB was associated with time interval after the QFT, but not with age.

Key messages

What is the key question?
- How good is the QuantiFERON-TB Gold In-Tube test (QFT) at predicting development of TB.

What is the bottom line?
- The QFT is widely used in TB low-incidence countries to screen for latent infection, but data on predictive values are limited.

Why read on?
- This is the largest study exploring the predictive values of the QFT in a TB low-incidence country, based on >50 000 person-years of follow-up.

INTRODUCTION

TB remains one of the leading causes of death and morbidity in the world, and despite recent advances in diagnosis and treatment, the task of controlling the disease seems monumental.[1]

More than a decade ago, interferon-γ release assays (IGRA) were introduced as tools to diagnose latent TB infection (LTBI), and they have a superior specificity, but a comparable sensitivity in patients with confirmed TB, compared with the widely used tuberculin skin test (TST).[2] IGRAs, such as the QuantiFERON-TB Gold In-Tube test (QFT), do not cross-react with the vast majority of non-tuberculous mycobacteria[3] or with the vaccination strain *Mycobacterium bovis* BCG.[4] The negative predictive value (NPV) is high in low-endemic countries, whereas the positive predictive value (PPV) of both the TST and the IGRAs generally is low.[5–8] Therefore, currently, only targeted testing in specific high-risk groups[9–12] is recommended.[13] Studies assessing the predictive value of IGRAs show heterogeneous results,[2 5 7 14] with a wide overlap between both PPV and NPV. Regarding the PPV, studies vary in design and population size and often the number of true incident cases is limited, all of which are factors compromising the generalisability.[2] Recently, WHO issued guidelines on the management of LTBI,[15] identifying the lack of studies on the risk of progression to active TB disease as a research gap.

In Denmark, QFT has been used since its introduction in 2005. Until 2010, the majority of tests has been performed in only one laboratory; the International Reference Laboratory of Mycobacteriology (IRLM) at Statens Serum Institut (SSI), Copenhagen. Similarly, all TB cases diagnosed in Denmark have been registered in one register at the Department of Infectious Disease Epidemiology (DIDE) at SSI, providing a unique opportunity to assess the association between QFT and subsequent TB.

The aim of this retrospective study was to determine the PPV and NPV of QFT for TB in a large Danish cohort comprising 15 980 individuals, and to identify potential factors associated with increased risk of TB.

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MATERIALS AND METHODS

Design

The study is a 5-year nationwide retrospective cohort study assessing the risk of TB among individuals with positive and negative QFT results. From January 2005 through December 2010, information on the first QFT result performed in Denmark as well as epidemiological data such as age, sex, date of active TB treatment initiation, and the result of diagnostic tests was retrieved from IRLM and DIDE. Incidence rates (IRs), PPVs and NPVs were determined and factors such as age and time since testing were assessed as possible risk factors for TB. For the subgroup of patients who developed incident TB, we collected additional data from hospital case records regarding immunosuppression, preventive chemotherapy, ethnicity and history of TB exposure. Patients were considered immunosuppressed if they were HIV positive, on treatment with tumour necrosis factor (TNF)-α inhibitors, receiving >20 mg/day prednisolone in combination with other disease-modifying antirheumatic drugs or on chemotherapy at the time of the QFT.

Study population

From 1 January 2005 to 31 December 2010, a total of 15 980 individuals had a QFT performed at IRLM, and from 1 January 2005 until end of follow-up 31 December 2012, 3123 patients were notified with TB to DIDE.

Of the 3123 TB cases, 271 had a QFT performed before TB treatment initiation and were included in the study. Recently, we reported the sensitivity of the QFT in the group of patients with active TB as well as QFT results for the whole population.[16] However, the value of QFT for predicting active TB in this population has not previously been assessed. Among persons who had a QFT performed and subsequently developed TB, the incidence of TB together with PPV and NPV were determined. We were unable to determine the exact reason for QFT testing for individual cases, but in Denmark, testing is recommended for (1) contact tracing, (2) screening before initiating immunosuppressive treatment and (3) sometimes as part of the investigations to diagnose TB.[17] In order to differentiate coprevalent or early TB from reactivation of LTBI, we stratified the study population into two groups: (a) TB diagnosed 0–90 days after the QFT was performed was considered coprevalent and (b) TB diagnosed >90 days after the QFT was considered incident TB in line with previous studies.[18 19] Rates of incident TB were calculated independently of the prescription of preventive treatment.

Diagnosis of TB

The diagnosis of TB was based on positive cultures, PCR and/or microscopies or based on clinical criteria only. The definition of pulmonary TB was based on at least one positive specimen from the lungs or clinical notification of pulmonary TB based on abnormal chest X-ray, whereas extrapulmonary TB was defined as TB found or notified from extrapulmonary site(s). All individuals were followed-up for 2 years or more until 31 December 2012.

QFT analyses

The QFT assay (Cellestis/Qiagen, Carnegie, Australia) was used. In brief, heparinised whole blood was incubated for 16–24 h in precoated tubes within 16–18 h from sampling. The QFT tubes were precoated with a mixture of synthetic *Mtb*-specific peptides representing ESAT-6, CFP-10 and TB7.7 as test antigens, phytohaemagglutinin (positive (mitogen) control) or saline (negative (nil) control). The concentration of interferon (IFN)-γ in the plasma was determined using the recommended ELISA test kit. QFT results were calculated using software provided by the manufacturer.

Ethics and approval

The project was approved by the Danish Data Protection Agency (Jrnl. no. 2011-54-1230), the Danish Health and Medicines Authority (3-3013-591/1) and reported to the regional health research ethics committee in the Capital Region of Denmark (protocol: H-1-2012-FSP90), who decided that additional ethical approval was not required.

Statistical analysis

Baseline patient characteristics were expressed as median and IQR (continuous variables) and as numbers and percentages (categorical variables). Wilcoxon rank-sum test was used to evaluate continuous variables and χ^2 test or Fisher's exact test was used to compare categorical variables. TB-free survival by initial test result was assessed using Kaplan–Meier estimates. Log-rank test was used to analyse Kaplan–Meier curves. The χ^2 test for trend in proportions was used to evaluate trend in predictive values across age groups and to evaluate time from QFT testing to development of incident TB. NPV for progression was defined as the proportion of study participants who scored negative for LTBI in the QFT and who did not develop TB during longitudinal follow-up. PPV for progression was defined as the proportion of patients that, given test positivity, subsequently developed active TB. A two-sided $p<0.05$ was considered to be significant.

RESULTS

Of the QFT results from 15 980 persons, 84.2% (13 463) were negative, 10.7% (1703) were positive and 5.1% (814) were indeterminate. The median follow-up time was 3.36 years (IQR 2.40–4.10), whereas the total follow-up person-years (PY) were 52 807. In total, 271 were diagnosed with TB, 231 with coprevalent TB and 40 with incident TB.

Among 1703 individuals with a positive QFT, 10.7% (183) and 1.32% (20) were diagnosed with coprevalent and incident TB, respectively (figure 1). Among 13 463 individuals with a negative QFT, 0.3% (38) and 0.15% (20) were diagnosed with coprevalent and incident TB, respectively. Among the 814 patients with indeterminate results, 10 cases of coprevalent TB and no cases of incident TB were found. There was no difference between cases with incident TB or no TB with respect to median age, sex or proportion of indeterminate results (table 1).

Association between QFT, age and coprevalent TB

Among 231 cases with coprevalent TB, 183 (79.2%) had positive, 38 (16.5%) had negative and 10 (4.3%) had indeterminate QFT results prior to the TB diagnosis (table 1). The relative risk (RR) between a positive and a negative QFT was 38.1 (CI 27 to 54). The risk of coprevalent TB was increased with age <35 years; the proportion of QFT-positive individuals <35 years who were diagnosed with TB was 19.3% compared with adults ≥35 years 7.2% (RR=2.7 (CI 2.1 to 3.5), $p<0.001$). The majority (>50%) of the coprevalent cases were diagnosed within 1 week after the QFT was performed (table 1).

Association between QFT, time, age and incident TB

Within the observation period, 40 patients developed incident TB among whom 20 (50%) had a positive, 20 (50%) had a negative and 0 had an indeterminate QFT result >90 days before TB

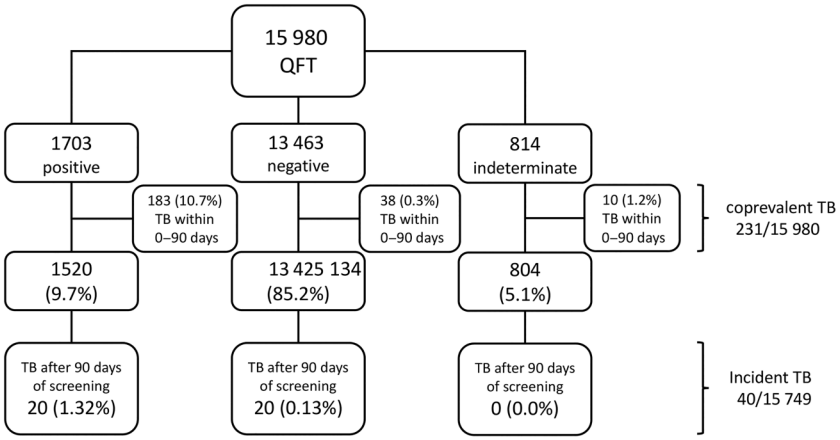

Figure 1 Flow chart of test results and occurrence of coprevalent and incident cases of TB after a QuantiFERON-TB Gold In-Tube test (QFT) performed. Coprevalent cases are defined as TB cases within 0–90 days after QFT, and incident TB cases as cases detected after 90 days from screening.

diagnosis. Based on data from hospital case records, 38% were of Danish origin, 43% had a history of MTB exposure, 3% were known HIV positive and 15% were immunosuppressed at the time of QFT. The majority of the incident TB cases were culture confirmed (73%) and 60% had pulmonary TB (tables 2 and 3). These proportions did not differ according to QFT result. Of the 20 cases with a positive QFT who developed incident TB, two had received isoniazid (INH) preventive therapy. They were aged 34 and 42 years, respectively, both treated due to recent exposure and both had TB with a fully susceptible MTB strain. Among the 18 cases that had not been offered treatment, 8 were younger than 35 years, and no reasons were given for not providing preventive therapy (tables 2 and 3).

We calculated a PPV and NPV for TB among the 15 749 individuals excluding the 231 coprevalent cases. PPV and NPV were 1.32% and 99.85%, respectively (table 4). The IRs were 383 per 10^5 PY (CI 241 to 603) and 45 per 10^5 PY (28–71), respectively, with an incidence rate ratio of 8.5. Figure 2 depicts the risk of developing incident TB according to QFT result in the study period. The median time from QFT to diagnosis of incident TB was 1.07 years (IQR 0.54–1.88). Among the QFT-positive cases, most of the incident TB cases (90%, 18/20) occurred within the first 2 years after the QFT with an IR of 792 per 10^5 PY (430–1422) for the first year, 402 per 10^5 PY (164–921) for the second year and 90 per 10^5 PY (16–364) after >2 years (p<0.001, test for trend and p=0.0026 when comparing IR in the first 2 years compared with IR after 2 years). Among the 20 QFT-negative incident TB cases, the development of TB was not associated with time from QFT (test for trend p=0.281).

The PPV was 2.22% among individuals <35 years compared with 0.99% among those aged ≥35 years, but this difference did not reach significance (p=0.087, table 4). Similarly, among the QFT-negative patients we found no influence of age above or below 35 years on the risk of developing TB (p=0.134, table 4).

Association between TB and median IFN-γ response

Among the 1703 QFT-positive individuals, 183 had coprevalent and 20 developed incident TB. The median IFN-γ levels were 4.73 (IQR 1.69–10.00) and 7.59 (1.34–10.00) IU/mL, respectively, and at a higher level than those who did not progress to TB 2.17 (0.74–7.98, p=0.027) (table 1). Six out of the 40 patients (15%) who developed incident TB (table 3) and 46 out of 231 (20.0%) with coprevalent TB had an IFN-γ level in the grey zone between 0.20 and 1.00 IU/mL.[20] In comparison, among those who did not develop TB, the proportion in the grey zone was significantly lower, 853/15 709 (5.4%, data not shown, p=0.02).

DISCUSSION

The present study is a large nationwide retrospective cohort study assessing the PPV of the QFT based on 15 980 persons tested with QFT with a follow-up time of ≥2 years. Our data confirm previous observations that in TB low-endemic

Table 1 Baseline characteristics

	No TB (n=15 709)	Coprevalent TB (n=231)	Incident TB (n=40)	p Value*
Median age years (IQR; range)	46 (33–60; 0–100)	37 (29–56; 0–85)	43 (34–53; 3–76)	0.394
Sex male, n (%)	7408 (47)	131 (57)	22 (55)	0.404
Culture/PCR confirmed, n (%)	NA	159 (69)	29 (73)	NA
Time from QFT to TB diagnosis (median weeks (IQR))	NA	1.0 (0.4–4.0)	55.6 (28.1–97.8)	NA
QFT result				
Indeterminate, n (%)	804 (5.1)	10 (4.3)	0 (0)	0.268
Negative, n (%)	13 405 (85.3)	38 (16.5)	20 (50)	NA
Positive, n (%)	1500 (9.6)	183 (79.2)	20 (50)	NA
Median IFN-γ IU/mL (IQR) For QFT-positive cases only	2.17 (0.74–7.98)	4.73 (1.69–10.00)	7.59 (1.34–10.00)	0.027

*p Comparison between no TB and incident TB using $\chi 2$ or Fisher's exact test for categorical variables and Wilcoxon rank-sum test for continuous variables.
Coprevalent cases are defined as TB cases within 0–90 days after QFT, and incident TB cases as cases detected after 90 days from screening.
QFT, QuantiFERON-TB Gold In-Tube test ; IFN, interferon; NA, not applicable.

Table 2 Summary characteristics of patients with incident TB, n=40

	QFT-positive (20)	QFT-negative (20)
Time from QFT to TB diagnosis (median weeks (IQR))	62 (32–86)	89 (36–132)
Age in years, (median (IQR))	37 (26–45)	52 (42–60)*
Male sex, n=22 (% male)	10/20 (50)	12/20 (60)
Danish ethnicity, n=15 (38%)	3/20 (15)	12/20 (60)†
Chemoprevention, n=2 (5%)	2/20 (10)	0/20 (0)
Immunosuppressed at QFT test time, n=7 (18%)	2/20 (10)	5/20 (25)
Mycobacterium tuberculosis exposure‡, n=13 (43%)	8/17 (47)	5/13 (38)
Culture/PCR confirmed TB, n=29 (73%)	15/20 (75)	14/20 (70)
Pulmonary TB, n=24 (60%)	10/20 (50)	14/20 (70)

*p<0.005.
†p=0.008.
‡Within the last 5 years, n=3 with a positive test had no exposure information available in patient files, n=7 with a negative test had no exposure information available.
QFT, QuantiFERON-TB Gold In-Tube test.

countries, a negative QFT has a very high NPV and a low but varying PPV.[5 21] Among persons with a positive QFT, age <35 years was associated with increased risk of being diagnosed with coprevalent TB and short time (<2 years) since the QFT performed was associated with subsequent diagnosis of incident TB.

A recent study by the Tuberculosis Network European Trials Group (TBNET) consortium, which investigated incident TB in contacts, defined as new TB cases diagnosed 84–968 days after QFT was performed, found a PPV and NPV of 1.9% (95% CI 1.1% to 3.0%) and 99.9% (95% CI 99.7% to 100%),[5] respectively, which is close to our findings of PPV 1.32% (95% CI 0.8% to 2.1%) and NPV 99.85% (95% CI 99.8% to 99.9%).

The IR for incident TB in the present study of 383 per 10^5 PY in QFT-positive and 45 per 10^5 PY in QFT-negative patients was in the low end compared with data from two meta-analyses by Rangaka *et al*[7] and Pai *et al*.[2] Rangaka *et al*[7] reported IR from 400 to 4800 per 10^5 PY for IGRA-positive cases and 200 to 2400 per 10^5 PY for IGRA-negative cases and Pai *et al*[2] reported IR from 370 to 8450 per 10^5 PY and 200 to 3200 per 10^5 PY, respectively in their meta-analysis, indicating a wide overlap between both PPV and NPV. Thus, despite high specificity of the IGRA the diagnostic accuracy is low. Other studies assessing the

Table 3 Characteristics of 40 patients who developed incident TB during follow-up

QFT result	Time since QFT (weeks)	Age	Sex	Country of origin	Prophylactic	Immunosuppressed	QFT (IU/mL)	Diagnosis	TB type
Positive	14	23	M	Turkey	No	No	1.27	Culture	PTB
	21	3	F	Somalia	No	No	10.00	PCR	EPTB
	22	43	M	Pakistan	No	No	7.05	Culture	EPTB
	25	34	M	Sri Lanka	Yes	Yes	8.13	Culture	EPTB
	29	32	F	Somalia	No	No	3.66	Culture	EPTB
	33	24	F	Somalia	No	No	1.34	Culture	EPTB
	43	26	F	Pakistan	No	No	10.00	No culture	EPTB
	47	43	F	Denmark	Yes	Yes	5.04	Culture	PTB
	52	25	M	Somalia	No	No	9.28	No culture	EPTB
	59	33	F	Vietnam	No	No	10.00	PCR	EPTB
	64	45	M	Somalia	No	No	10.00	Culture	PTB
	64	76	M	Denmark	No	No	0.52	No culture	PTB
	80	21	M	Somalia	No	No	10.00	No culture	EPTB
	83	44	F	Greenland	No	No	10.00	Culture	PTB
	86	58	F	Vietnam	No	No	10.00	Culture	EPTB
	86	45	M	Denmark	No	No	0.74	Culture	PTB
	90	38	F	Unknown	No	No	10.00	No culture	PTB
	98	49	M	Greenland	No	No	1.32	Culture	PTB
	174	35	M	Greenland	No	No	0.57	Culture	PTB
	242	46	F	Greenland	No	No	1.48	Culture	PTB
Negative	14	61	F	Pakistan	No	No	0.00	Culture	EPTB
	16	52	F	Ghana	No	No	0.00	No culture	PTB
	18	34	F	Uganda	No	No	0.08	No culture	PTB
	20	61	M	Denmark	No	No	0.00	No culture	EPTB
	23	40	F	Bangladesh	No	No	0.10	PCR	EPTB
	40	51	M	Unknown	No	No	0.00	Culture	EPTB
	45	55	M	Denmark	No	No	0.00	No culture	PTB
	47	62	M	Denmark	No	Yes	0.00	No culture	PTB
	50	52	M	Denmark	No	HIV	0.06	Culture	PTB
	80	51	F	Denmark	No	No	0.25	Culture	PTB
	98	50	M	Somalia	No	No	0.00	Culture	PTB
	103	59	F	Sri Lanka	No	No	0.00	Culture	EPTB
	113	40	M	Somalia	No	No	0.30	Culture	EPTB
	129	42	M	Denmark	No	Yes	0.01	Culture	PTB
	130	57	F	Denmark	No	Yes	0.00	Culture	PTB
	140	62	M	Denmark	No	No	0.09	Culture	PTB
	143	75	F	Denmark	No	Yes	0.00	No culture	PTB
	148	39	M	Denmark	No	No	0.03	Culture	PTB
	159	23	M	Denmark	No	No	0.24	Culture	PTB
	161	43	M	Denmark	No	No	0.00	Culture	PTB

EPTB, extrapulmonary TB; PTB, pulmonary TB; QFT, QuantiFERON-TB Gold In-Tube test.

Table 4 Development of incident TB according to age groups

Age (years)	Total N	TB (n)	PPV/NPV %	PY*	IR†	95% CI (10^3)	IRR‡
QFT-positive							
<15	52	1	1.92	177	565	(0.3 to 35.9)	NA
15–24	118	3	2.54	402	746	(1.9 to 23.5)	29.8
25–34	236	5	2.12	764	654	(2.4 to 16.1)	43.6
<35 total	*406*	*9*	*2.22*	*1343*	*670*	*(3.3 to 13.2)*	*41.9*
≥35	1114	11	0.99	3875	284	(1.5 to 5.2)	5.1
Total	1520	20	1.32	5217	383	(2.4 to 6.0)	8.5
QFT-negative							
<15	594	0	100	1809	0	(0.0 to 2.6)	NA
15–24	1248	1	99.92	4002	25	(0.0 to 1.6)	NA
25–34	1938	1	99.95	6488	15	(0.0 to 1.0)	NA
<35 total	*3780*	*2*	*99.95*	*12 299*	*16*	*(0.0 to 0.7)*	*NA*
≥35	9652	18	99.81	32 280	56	(0.3 to 0.9)	NA
Total	13 425	20	99.85	44 579	45	(0.3 to 0.7)	NA

*PY of follow-up.
†IR per 10^5 PY of follow-up.
‡IRR calculated for groups of 'QFT-positive' and 'QFT-negative'.
χ^2 test for trend in proportions (cases/PY) between QFT-positive and age groups: p=0.086. χ^2 comparison of proportions among QFT-positive above or below 35: p=0.087. χ^2 test for trend in proportions among QFT-negative and age groups: p=0.105. χ^2 comparison of proportions among QFT-negative above or below 35: p=0.134.
IR, incidence rate; IRR, incidence rate ratio; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value; PY, person-years; QFT, QuantiFERON-TB Gold In-Tube test.

predictive value in a low-endemic setting have found PPV of IGRA ranging from 0.8% in Portuguese healthcare workers[22] and 2.4% among Dutch contacts who did not start preventive therapy[8] to approximately 7%–8% in HIV positive[10 23] to 28.6% in recently exposed children.[21] Generally, above studies are limited by low number of incident TB cases ranging from 2 to 24 cases and variable design and follow-up periods.[5 6 10 18 19 21 23] We have identified 40 incident cases in minimum 2 years of follow-up time, which makes this one of the largest cohorts studied. The IR for incident TB among the QFT-negative of 45 per 10^5 (28–71) PY was higher than in general for the Danish population (6.3 per 10^5 PY), most likely because QFT testing is used in persons with a higher a priori risk of TB. Furthermore, two-thirds of all TB cases in Denmark develop among foreign born, where the incidence of TB is 39.8 per 10^5 PY.[24]

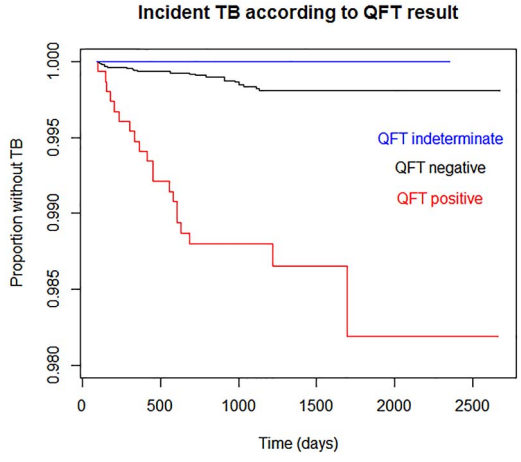

Figure 2 Kaplan–Meier plot of incident TB illustrates the proportion of TB-free survival as a function of follow-up time for a negative and positive QuantiFERON-TB Gold In-Tube test (QFT) (p comparison of curves using log-rank test p<0.000). Furthermore, a plot for indeterminate results is shown.

Among all screened individuals, the overall rate of persons with a positive QFT was 10.7% (1703/15 980). In a recent major review, Getahun *et al*[9] assessed the prevalence of latent TB in different risk groups, and found in TB low-incidence populations the positivity rate to vary substantially from 4% to 23% in anti-TNF candidates, 1% to 19% in healthcare workers, 10% to 54% in recent immigrants from high-incidence settings and 7% to 55% in close contacts with recent exposure.

Time since test

It is well known that the risk of TB declines with time since exposure[25] and recently exposed individuals with a QFT conversion are reported to have an approximately eightfold higher risk of progression to TB disease within 2 years.[26] We found that 90% (18/20) of the cases with a positive baseline QFT who developed incident TB did so within 2 years after testing, suggesting a very limited risk of progression to active disease after >2 years after testing. For cases with an initial negative QFT who developed TB, we found that 35% (7/20) developed TB >2 years after the initial negative QFT. Whether this could be explained by low sensitivity of the initial negative QFT (false negative) or due to new infection is not possible to determine.[5 21]

Interferon-γ

Among those with a positive QFT, the level of IFN-γ was significantly higher among those who were later diagnosed with TB. It has been debated, whether the magnitude of the IFN-γ response can serve as a prognostic marker for subsequent progression to active disease and discriminate between those who will progress and those who will not. Animal studies have shown that active TB was preceded by an increase in *Mycobacterium tuberculosis*-specific IFN-γ,[27 28] in humans, however, this relation is controversial. Diel *et al*[21] found significantly higher IFN-γ values among contacts who progressed to TB suggesting that high levels of IFN-γ can serve as a prognostic marker for subsequent development of active disease,[29 30] but there was wide overlap between initial IFN-γ levels among incident TB cases and those that remained healthy, indicating a low prognostic accuracy. Other studies found no differences in median IFN-γ response in individuals who developed TB compared with those who did not.[5 31] Algorithms or new antigens that could identify those at highest risk of progression are still needed.[32]

Age

We found that the risk in QFT-positive individuals of being diagnosed with coprevalent TB was significantly higher for persons <35 years. This association is not surprising, given the fact that children most often have a known recent exposure, whereas an adult more likely could have been remotely infected. For incident TB, such an association was neither significant in the trend test (0.086) nor in Kaplan–Meier curves and the log-rank test (p=0.055, figure 2). Children are known to be more vulnerable to TB,[9] but several factors may explain the lack of association with age for incident TB. First, 20 cases of incident TB developed among the QFT-positive persons, and given this relatively low number of cases our study is only powered to detect large differences in incident TB risk. Second, children and individuals aged <35 years at risk of TB are more likely to receive preventive treatment. Therefore, more cases would have

been prevented in the young age group, resulting in a lower risk of TB. Third, we believe that more children may have been diagnosed at an earlier stage with coprevalent TB rather than incident TB. This is supported by the fact that we saw an increased risk of coprevalent TB among those <35 years. Finally, most physicians would start anti-TB treatment in a child with a positive QFT and signs of TB, although the test in itself is not diagnosing active TB.

Preventive treatment

Only 2/20 patients with incident TB with a positive baseline QFT received preventive therapy, even though half were <35 years and thus belonged to a category of patients where preventive treatment is recommended.[33] There were no reasons given for not offering preventive therapy even though INH therapy has been shown to effectively reduce the risk of subsequent progression to TB.[5 6 9] Information on preventive treatment was not available for the majority of the population, but we estimate that the coverage of INH preventive therapy is around 35% in Denmark using data from DIDE and data on INH prescriptions in Denmark (data not shown) allowing room for improvement. In studies performed in neighbouring countries, adherence to guidelines is highly variable,[34 35] that is, in Germany, only 20% of IGRA-positive adult contacts received preventive therapy,[34] whereas 80% did so in a cohort of asylum seekers in Switzerland.[35] Our data suggest it could be relevant to reinforce current guidelines for preventive therapy in Denmark.

Limitations and strengths

The study was retrospective and registry based. Thus, we did not have information on the actual reason for IGRA testing and on INH preventive treatment. However, our cohort is large and includes data for every person in Denmark who had a QFT performed and every person who subsequently was diagnosed with TB within a follow-up period of at least 2 years and up to 7 years.

Most likely, our data underestimate the real prognostic value of the QFT for several reasons. In Denmark, the QFT is typically used in three different situations (a) for contact tracing, (b) before immunosuppression and (c) where active disease is suspected. Individuals screened before immunosuppressive therapy will tend to have lower risk of developing TB as preventive therapy is provided as part of the recommended management of patients receiving TNF-α inhibitors as specified in Danish guidelines.[36] Thus, treatment for LTBI in this group of patients would tend to reduce the incidence of TB and thus underestimate the PPV. We did not include information on deaths during the study period, thus our calculations of follow-up time in cases who did not develop TB could be falsely high, which again may result in underestimating the PPV. In addition, we did not have information on immigration and the number of cases who developed TB outside Denmark. This could affect the PPV in both directions. The NPV would be underestimated when cases with an initial negative QFT subsequently become infected.

In summary, among individuals with a positive QFT, we found a high proportion of cases with coprevalent TB (10.7%), especially among those <35 years (19.3%). The PPV for incident TB was 1.3% and the IR was highest within the first 1–2 years of testing. We confirm that a negative QFT has a very high NPV in TB low-endemic countries. We recommend more intensified diagnostic investigations in individuals with a positive QFT within the first 2 years after testing. In addition, guidelines for preventive treatment for recently infected individuals (<2 years) should be reinforced.

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Competing interests TSH and TL are working at Statens Serum Institut in a department that analyses QuantiFERON tests from parts of Denmark; however, they declare no relationship or other associations that might pose a conflict of interest (eg, pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents or research funding); PR declares that Hvidovre Hospital has filed a patent on the use of IP-10 as a marker for infection with mycobacterium TB and PR is one of the registered coinventors; PR has been invited speaker by Cellestis and has received QFT-IT kits at a reduced price for non-profit research.

Ethics approval The project was approved by the Danish Data Protection Agency (Jrnl. no. 2011-54-1230), the Danish Health and Medicines Authority (3-3013-591/1) and reported to the regional health research ethics committee in the Capital Region of Denmark (protocol: H-1-2012-FSP90), who decided that additional ethical approval was not required.

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