Stratification by interferon gamma release assay level predicts risk of incident tuberculosis.

Corresponding author:
Brita Askeland Winje, Department of Vaccine Preventable Diseases, Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, 0403 Oslo, Norway. E-mail: brita.winje@fhi.no, telephone 47 21076853

Authors:
Richard White, Department of Infectious Disease Epidemiology and Modelling, Norwegian Institute of Public Health, Oslo, Norway, E-mail: Richard.White@fhi.no

Heidi Syre, Department of Medical Microbiology, Stavanger University Hospital, Stavanger, Norway, E-mail: heidi.syre@sus.no

Dag Harald Skutlaberg, Department of Microbiology, Haukeland University Hospital, Bergen, Norway and Department of Clinical Science, University of Bergen, Bergen, Norway, E-mail: dag.harald.skutlaberg@helse-bergen.no

Fredrik Oftung, Department of Infectious Disease Immunology, Norwegian Institute of Public Health, Oslo, Norway, Email: Fredrik.Oftung@fhi.no

Anne Torunn Mengshoel, Department of tuberculosis, blood borne and sexually transmitted infections, Norwegian Institute of Public Health, Oslo, Norway, E-mail: anne.torunn.mengshoel@fhi.no

Hege Salvesen Blix, Department of Pharmacoepidemiology, Norwegian Institute of Public Health, Oslo, Norway and School of Pharmacy, University of Oslo, Norway, E-mail: hege.salvesen.blix@fhi.no

Arne Broch Brantsæter, Department of Infectious Diseases and Department of Acute Medicine, Oslo University Hospital, Oslo, Norway, E-mail: UXARBR@ous-hf.no

Ellen Kristine Holter, Department of Microbiology, Oslo University Hospital, Oslo, Norway, E-mail: eholter@ous-hf.no

Nina Handal, Department of Microbiology and Infection control, Akershus University Hospital, Lørenskog, Norway, E-mail: Nina.Handal@ahus.no

Gunnar Skov Simonsen, Department of Microbiology and Infection Control, University Hospital of North Norway, and Research Group for Host-Microbe Interaction, Faculty of Health Sciences, UiT-The Arctic University of Norway, Tromsø, Norway, E-mail: Gunnar.Skov.Simonsen@unn.no

Jan Egil Afset, Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, and Department of Medical Microbiology, St. Olavs Hospital, Trondheim, Norway, E-mail: jan.afset@ntnu.no

Anne Marte Bakken Kran, Department of Microbiology, Oslo University Hospital, Oslo, Norway and Institute of clinical medicine, Faculty of Medicine, University of Oslo, Norway E-mail: a.m.b.kran@medisin.uio.no

ABSTRACT

Introduction Targeted testing and treatment of latent tuberculosis infection (LTBI) are priorities on the global health agenda, but LTBI management remains challenging. We aimed to evaluate the prognostic value of the QuantiFERON® TB-Gold (QFT®) test for incident tuberculosis (TB), focusing on the interferon (IFN)-γ level, when applied in routine practice in a low TB incidence setting.

Methods In this large population-based prospective cohort, we linked QFT® results in Norway (1 Jan 2009–30 June 2014) with national registry data (Norwegian Surveillance System for Infectious Diseases, Norwegian Prescription Database, Norwegian Patient Registry, and Statistics Norway) to assess the prognostic value of QFT® for incident TB. Participants were followed until 30 June 2016. We used restricted cubic splines to model non-linear relationships between IFN-γ levels and TB, and applied these findings to a competing risk model.

Results The prospective analyses included 50,389 QFT® results from 44,875 individuals, of whom 257 developed TB. Overall, 22% (n = 9878) of QFT® results were positive. TB risk increased with the IFN-γ level until a plateau level, above which further increase was not associated with additional prognostic information. The hazard ratios for TB were 8.8 (95% CI 4.7-16.5), 19.2 (95% CI 11.6-31.6) and 31.3 (95% CI 19.8-49.5) times higher with IFN-γ levels of 0.35 to <1.00, 1.00 to <4.00 and ≥ 4.00 IU/ml, respectively, when compared with negative tests (<0.35 IU/ml).

Conclusions Consistently, QFT® demonstrates increased risk of incident TB with rising IFN-γ concentrations, indicating that IFN-γ levels may be used to guide targeted treatment of LTBI.

Summary box

- **What is the key question?** Does stratification by level of interferon (IFN)-γ measured by QuantiFERON TB-Gold® add prognostic value when assessing risk of incident tuberculosis?

- **What is the bottom line?** In this largest cohort to date, we found that higher levels of IFN-γ were associated with consistently greater risk of incident TB.

- **Why read on?** Our findings indicate that IFN-γ levels may be used to guide targeted treatment of LTBI

Keywords: QuantiFERON® TB-Gold, interferon-gamma release assay, latent tuberculosis, tuberculosis

Word count: 3885

INTRODUCTION

Targeted testing and treatment of latent tuberculosis infection (LTBI) are important components of the World Health Organization's (WHO's) End TB strategy in low-incidence countries.[1-3] The overall tuberculosis (TB) incidence rate (IR) in Norway is 6 per 100,000 population per year.[4] Foreign-born individuals account for almost 90% of TB notifications and carry an almost 70-fold higher risk of TB IR (42/100,000) compared with the Norwegian-born population, in which the TB IR (0.6/100,000) has reached the pre-elimination phase.[4] Although sporadic outbreaks occur, routine molecular surveillance for *Mycobacterium tuberculosis* strains confirms the overall low TB transmission rate.[4] Against this backdrop, screening and preventive treatment of LTBI has gained high priority in Norwegian TB control activities. A well-established, mandatory screening programme for TB and LTBI targets (i) immigrants arriving from countries with high TB incidences, (ii) pre-employment screening in selected groups (health care workers and those working with children), and (iii) other groups at increased risk of TB, specifically contacts.

However, LTBI screening and treatment remain challenging, due partly to the suboptimal nature of diagnostic tests.[2] The traditional tuberculin skin test (TST) and the more recently introduced interferon gamma release assays (IGRAs) are both indirect markers of TB infection, indicating a cellular immune response to the *M. tuberculosis* complex.[2] In 2009, Norway introduced the QuantiFERON® TB-Gold (QFT®; Qiagen, Hilden, Germany) IGRA as a confirmatory test for use in individuals with TST positivity on routine screening (≥ 6 mm, obtained with the Mantoux method using purified protein derivate, RT 23, 2 TU; Statens Serum Institute, Copenhagen, Denmark). Initial expectations were high because of improved specificity of QFT® compared with the TST as antigens included in the QFT® are not encoded in the genome of *M. bovis* (bacillus Calmette-Guérin, BCG) or most non-tuberculous mycobacterial strains.[5] However, several diagnostic challenges remain, including poor reproducibility, definition of a single cut-off value for a positive test, and difficulty of interpreting low positive results.[2] Furthermore, QFT® does not distinguish among the various stages evolving from latent infection to TB disease, or reactivation from re-infection, which renders its prognostic value questionable.[2]

The aim of this study was to evaluate the prognostic value of the QFT® for incident TB when applied in routine practice in a low-TB-incidence country. We present data from a large prospective cohort of individuals tested with the QFT®. We focused specifically on the interferon (IFN)-γ level and the significance of low positive results.

METHODS

In this nation-wide population-based prospective cohort, QFT® results were linked with data from high-quality national population-based registers using 11-digit personal identification numbers. All eight laboratories performing the QFT® in Norway during the study period (1 January 2009–30 June 2014) provided QFT® data. Of 77,812 QFT® results provided, 27,423 (35%) were excluded for the following reasons: (i) lack of a valid identification number, preventing linkage to health data (*n* = 14,903); (ii) not possible to extract information electronically on the IFN-γ value from two of the laboratory databases (*n* = 11,774); and (iii) TB diagnosis before or within 3 months after QFT® testing (n=746). Thus 50,389 QFT® from 44,875 individuals were included for prospective analyses (figure 1).

<Insert Figure 1 about here>

We obtained demographic data from Statistics Norway, TB notifications and prescriptions for LTBI treatment from Norwegian Surveillance System for Infectious Diseases (MSIS), outpatient drug prescriptions from Norwegian Prescription Registry (NORPD), and hospital discharge data from Norwegian Patient Registry (NPR). Data linkage was last updated in June 2016 (≥2 years after last QFT® test).

Data management

We only had data on time of QFT® by month and year, and we could not ascertain the chronological order of QFT® results in 496 (1.1%) individuals tested twice in the same month and year. Among these, 131 had discordant results. For these individuals we selected conclusive over inconclusive test results (n=44), and by random order for the remaining (n=87).

Country of birth was dichotomised as Norwegian or foreign. This was not recorded for 445 (1%) individuals, who we designated as foreign-born, assuming that they were recent immigrants.

Classification of underlying diseases and outpatient immunosuppressive treatments was based on International Classification of Diseases, 10th revision[6] or the Norwegian Classification of Medical Procedures (NCMP)[6] for data from NPR, and Anatomical Therapeutic Chemical codes for data from NORPD[7] (Appendix 1). An underlying disease was included in the analysis as a risk factor when it was first recorded before or at the same time as QFT® test, and iatrogenic immunosuppression when at least one prescription (usually covering 3 months of treatment) was registered within 6 months before QFT® test. Included risk factors were consistent with those listed in national guidelines.[8]

Main outcome and exposure

Incident TB was the main outcome of interest (event). In the main analysis, we defined incident TB as sample collection for TB diagnosis >3 months after QFT® testing, in line with previous studies.[9] As some cases identified in this manner may represent co-prevalent TB, we conducted a sensitivity analysis with the threshold for incident TB set at 6 months after QFT® testing. The main exposure was the IFN-γ level (IU/ml) calculated according to the manufacturers recommendations.

Statistical analyses

We used STATA14 for statistical analysis. [10] The statistical approach is presented in detail in Appendix 2. Participants were followed until 30 June 2016. As the TB risk may change over time, and some individuals had more than one QFT®, we applied a Cox regression model with time-dependent covariates (for calculating hazard ratios) to examine associations between the main outcome and exposure. This involved constructing a row of data for each QFT®, from the start of the interval (date of sampling) until the end of the interval (event, censoring or date of sampling for a subsequent test). Covariate values are those that apply over that interval. Using time-varying explanatory variables is more robust than selecting exposures from a single time point as it utilizes all available data. As underlying disease and immunosuppressive treatment data were correlated strongly, they were combined to form an "any medical risk factor" covariate in the regression analysis. We ran a competing-risks model with emigration, death, or preventive treatment of LTBI serving as competing risks.

Splines and categorisation of IFN-γ levels

We had a priori information that the association between incident TB and IFN-γ levels was non-linear. Three laboratories reported continuous IFN-γ levels only until 10.0 IU/ml, with '≥10.0 IU/ml'

used for higher values. We thus modelled the continuous data using restricted cubic splines, to gain insight into appropriate categorisation of the data and to enable usage of all available results. Only tests with IFN-γ levels < 10.0 IU/ml were included in the spline models. We ran two regression analyses including origin, age, and identified medical risk factors as adjustment variables: one analysis had knots at 0.35, 3.0, and 6.0 IU/ml, and the other had knots at 0.35, 0.7, 2.0, 4.0, 6.0, and 8.0 IU/ml. The lowest knot values (0.35 and 0.7 IU/ml) were selected based on clinicians' input, and the remaining were based on equal spacing. Figure 2a had higher akaike information criterion (AIC) than Figure 2b, suggesting that Figure 2b fits the data better. However, results of both analyses supported the categorisation of IFN-γ levels as negative (<0.35 IU/ml, according to the manufacturer's cut-off value), low positive (0.35 to <1.0 IU/ml), medium positive (1.0 to <4.0 IU/ml), and high positive (≥4.0 IU/ml). We used these categories in all further analyses. We also ran regressions with the outcome restricted to culture confirmed incident TB.

Effect modification and interaction terms

We investigated whether the association between the IFN-γ level and incident TB was modified by country of origin, age, or identified medical risk factor using likelihood ratio tests. We found no significant interactions and thus did not include them in the regression models.

IRs, predictive values and numbers needed to treat (NNT)

We calculated IRs as the numbers of incident TB per 1000 person-years, and negative- and positive predictive values (NPVs/PPVs) separately for the first 2 years and for subsequent years. We also calculated predictive values for two hypothetical non-informative tests, in which we assumed that all test findings were negative (for the hNPV) and positive (for the hPPV) respectively. We calculated the average number of LTBI treatments needed to prevent one incident TB , by estimating the difference in risk of incident TB among individuals who did not and those who did receive LTBI treatment, NNT=1/(incident TB/number of individuals not receiving LTBI treatment –incident TB/individuals receiving LTBI treatment), with corresponding confidence limits.[11] These analyses were performed on the first QFT® (also in individuals with several tests) to avoid survival bias. Although treatment for LTBI was accounted for by censoring subjects, this does not account for clinicians selectively treating patients at highest risk. We can therefore interpret our outcome as "incident TB if not prevented by LTBI treatment".

RESULTS

Characteristics of the study population

The analysis included 50,389 QFT® results from 44,875 individuals. In total, 40,146 (89%) individuals had one, 4123 (9%) had two, and 606 (1%) had three or more QFT® tests (range, 1–8).

Table 1 presents the characteristics of the study population. The foreign-born population was younger, included more females, more often underwent the QFT® based on primary health care screening, and was less likely to have immunosuppressive conditions compared with the Norwegian-born population.

Table 1 Characteristics of the population included in prospective analyses at the time of QuantiFERON-TB Gold (QFT®) testing[a]

Baseline characteristic	Norwegian-born	Foreign-born	Total	*p*
Total population[b]	25,457 (57)	19,418 (43)	44,875 (100)	
Sex (male)	11,426 (45)	7941 (41)	19,367 (43)	<0.001
Age (years), median [IQR]	44 [26-60]	31 [25-40]	36 [25-53]	<0.001
Age group (years)				<0.001
<5	940 (4)	372 (2)	1312 (3)	
5–14	1600 (6)	1144 (6)	2744 (6)	
15–34	6651 (26)	10,452 (54)	17,103 (38)	
35–64	11,578 (46)	6810 (35)	18,388 (41)	
≥65	4688 (18)	640 (3)	5328 (12)	
Observation time after QFT® (months), median [IQR] [c]	43 [31-60]	43 [28-63]	43 [29-61]	<0.001
Health care level of QFT® request				<0.001
Primary health care (screening)	7004 (28)	11,736 (60)	18,740 (42)	
Outpatient hospital clinic	5982 (24)	4577 (24)	10,559 (24)	
Paediatric in-/outpatient unit	1179 (5)	471 (2)	1650 (4)	
Inpatient, internal medicine	3409 (13)	1391 (7)	4800 (11)	
DMARD-relevant medical unit[d]	7880 (31)	1242 (6)	9122 (20)	
Identified underlying disease, any[e]	13,774 (54)	2984 (15)	16,758 (37)	<0.001
HIV infection	295 (1)	610 (3)	905 (2)	
Diabetes	1376 (5)	704 (4)	2080 (5)	
Malignant neoplasm	1657 (7)	312 (2)	1969 (4)	
Chronic renal disease	589 (2)	180 (1)	769 (2)	
Solid organ transplant	140 (0.6)	46 (0.2)	186 (0.4)	
DMARD-relevant diagnosis[d]	11,498 (45)	1530 (8)	13,028 (29)	
Malnutrition	514 (2)	79 (0.5)	593 (1)	
Alcohol/opiate dependence syndrome	389 (1.5)	60 (0.3)	449 (1)	
Iatrogenic immunosuppression, any[f]	6311 (25)	634 (3)	6945 (15)	<0.001
Long-term glucocorticosteroids[g]	256 (1)	41 (0.2)	297 (0.6)	
Antineoplastic agents	1040 (4)	71 (0.4)	1111 (2)	
Selective immunosuppressants	539 (2)	57 (0.3)	596 (1)	
TNF-alpha inhibitors	925 (4)	78 (0.4)	1003 (2)	
Interleukin inhibitors	31 (0.1)	6 (0)	37 (0.1)	
Systemic calcineurin inhibitors	182 (0.7)	42 (0.2)	224 (0.5)	
Other immunosuppressants[h]	4072 (16)	412 (2)	4484 (10)	

Data are presented as n (%) or median [interquartile range].

[a]At time of first QFT® when several tests were administered.

[b]Demographic data were obtained from Statistics Norway.

[c]From time of QFT® until event (TB) or LTBI treatment, death, emigration, or study end.

[d]Includes rheumatological, dermatological, neurological, and gastroenterological medical units/diseases.

[e]Includes medical risk factors that were present prior to or at the time of QFT® testing. Sources for classifications are ICD-10/NCMP codes from the Norwegian Patient Registry and ATC codes from the Norwegian Prescription Database.

[f]Includes outpatient prescriptions for immunosuppressive treatment, obtained from the Norwegian Prescription Database. Prescriptions within 6 months prior to QFT® testing were included.

[g]Systemic corticoids ≥15 mg/day for ≥1 month.

[h]Methotrexate (L04AX03) accounted for 79% of prescriptions.

DMARD, disease-modifying anti-rheumatic drug; TNF, tumour necrosis factor.

In the foreign-born group, 7644 (39%) individuals were born in countries with WHO-estimated TB IRs > 200 per 100,000 population.[12]

Among the 17,103 (38%) individuals with at least one identified medical risk factor, 6600 (39%) had records of underlying disease and immunosuppressive treatment, 10,158 (59%) had records of underlying disease only, and 345 (2%) had records of immunosuppressive treatment only. Diagnoses relevant to DMARD treatment dominated medical risk factors.

QFT® results and incident TB

Overall, 22% (*n* = 9878) individuals had positive, 76% (*n* = 34,128) had negative, and 2% (*n* = 869) had inconclusive QFT® results (based on the first result in individuals with multiple tests). Among individuals with positive QFT® results, 2166 (22%) had IFN-γ levels < 1.00 IU/ml, of whom 1476 (68%) had levels < 0.7 IU/ml. Among individuals with an inconclusive first QFT® result, 303 (35%) were retested. Among them 249 (82%) yielded a conclusive result in which a majority were reported negative (n=222).

Incident TB was reported in 257 individuals [foreign-born, *n* = 229 (89%); Norwegian-born, *n* = 28 (11%)]. A total of 155 (60%) cases were confirmed by culture, of whom 86 (55%) were pulmonary TB. The median time from QFT® test to TB diagnosis was 9 months [IQR 5-19]. Incident TB occurred in 219 (2.2%) individuals with QFT® positivity, 33 (0.1%) individuals with QFT® negativity, and 5 (0.6%) individuals with inconclusive results. Fourteen of the 33 individuals with negative QFT® results and three of the five individuals with inconclusive QFT® results showed positivity on subsequent tests. Results of the sensitivity analysis for incident TB are presented in Appendix 3. Although the use of a 6-month threshold reduced the number of incident TB cases, associations between the main outcome and exposures were not affected.

Hazard ratios for incident TB

Figure 2 shows hazard ratios for incident TB by IFN-γ level separately for the two linear spline functions. Curve smoothing is greater for the three-knot spline (Figure 2a) than for the six-knot spline (Figure 2b), indicating that the latter captured more of the underlying variability. The hazard ratios for incident TB increased with the IFN-γ level up to 1.00–4.00 IU/ml, and then levelled off. Increases in the IFN-γ level above this point added little prognostic information to the hazard ratios for incident TB. The model with fewer knots had an AIC of 3128, and the model with more knots had an AIC of 3103, suggesting that the model with more knots fits the data better. Restricting the analysis to culture confirmed incident TB showed similar figures, although with wider confidence limits (Appendix 4)

<Insert Figure 2ab about here>

Table 2 presents hazard ratios from multivariable time-dependent Cox regression analysis for incident TB by IFN-γ level category, country of origin, and age. After the exclusion of individuals with inconclusive QFT® (*n* = 869, of whom five had incident TB), the analyses included 48,121 QFT® results from 44,006 individuals. The hazard ratios for TB were 8.8 (95% CI 4.7-16.5), 19.2 (95% CI 11.6-31.6) and 31.3 (95% CI 19.8-49.5) times higher in the low, medium and high IFN-γ positive categories, respectively, compared with a negative test.

Foreign-born status and age < 35 years were associated significantly with incident TB, regardless of IFN-γ level; no such association was found for the presence of at least one medical risk factor.

Table 2 Univariate- and multivariable time-dependent Cox regression results for incident tuberculosis (*n* = 252) by IFN-γ level, age group, country of origin and medical risk factors (*n* = 48,121) QuantiFERON TB Gold (QFT®) results for 44,006 individuals)

Covariate	No. of tests	TB events[a]	Years[b]	*c* HR	a HR	*p*	95% CI
IFN-γ level (IU/ml)[c]						<0.001[d]	
Negative (<0.35)	*37,253*	*29*	*133,647*	*1 (ref)*	*1 (ref)*		
Low positive (0.35 to <1.0)	2488	16	6995	10.7	8.8	<0.001	4.66-16.50
Medium positive (1.0 to <4.0)	2971	50	9087	25.1	19.2	<0.001	11.62-31.60
High positive (≥4.0)	5373	157	16,233	43.0	31.3	<0.001	19.82-49.53
Origin							
Foreign-born	*21,016*	*224*	*71,983*	*1 (ref)*	*1 (ref)*		
Norwegian-born	27,105	28	94,045	0.09	0.6	0.015	0.36–0.90
Age group (years)							
≥35	*25,381*	*83*	*88,163*	*1 (ref)*	*1 (ref)*		
<35	22,740	169	77,865	2.3	1.6	0.001	1.23–2.14
Any medical risk factor[e]							
None	*29,674*	*221*	*104,063*	*1 (ref)*	*1 (ref)*		
At least one	18,447	31	61,964	0.3	1.3	0.234	0.84–2.03

[a]Diagnosed > 3 months after QFT® testing.
[b] Sum of person-years of follow-up after QFT® testing.
[c]Denominators vary due to missing IFN-γ levels.
[d]Likelihood-ratio test for the whole IFN-γ level variable.
[e]Based on ICD-10/NCMP codes for data from the Norwegian Patient Registry and ATC codes for data from the Norwegian Prescription Database.
IFN, interferon; QFT®, QuantiFERON-TB Gold; TB, tuberculosis; *c*HR, crude hazard ratio; *a*HR, adjusted hazard ratio CI, confidence interval.

TB IRs, incidence rate ratios (IRRs) and predictive values

The overall rate of incident TB in the study population was 1.52 per 1000 person-years. TB IRs varied greatly depending on QFT® results, time since testing, age, and country of origin (Table 3). Eighty percent (*n* = 205) of incident TB cases occurred within 2 years after QFT® testing, with corresponding higher IRs (definers) for TB in this period compared with the subsequent period in all groups. The IRR (definer) for incident TB following a positive QFT® result was 4.4, when comparing the first 2 years with subsequent years. IRs increased with the IFN-γ level category.

IRs following QFT® positivity varied by age. Few incident TB events occurred in the youngest and oldest age groups. PPVs varied, but were low in all groups (0.1–4.5%), and NPVs were high (>99%; Table 3). The QFT® added very little predictive value compared with the hypothetical non-informative test in this population.

Table 3 Incidence rates and predictive values for incident TB from the time of QuantiFERON TB-Gold (QFT®) testing, by observation time (</≥2 years) and IFN-γ category[a]

QFT® result	*N*	Years	TB	IR	PPV [hPPV][b]	NPV [hNPV][b]
<2 years after QFT®(or until TB, LTBI treatment, death, emigration, or study end)						
Total study population	44,875	80,270	205	2.6	[0.5]	[99.5]
Positive, total	9 878	15,802	176	11.1	1.8	-
Low positive	2166	3593	14	3.9	0.6	-
Medium positive	2670	4259	38	8.9	1.4	-
High positive	5042	7950	124	15.6	2.5	-
Negative	34128	63,064	24	0.4	-	99.9
Inconclusive	869	1404	5	3.6	-	-
Positive, by age (years)						
<5	66	73	3	41.2	4.5	-
5–14	376	396	9	22.7	2.4	-
15–34	5293	8371	111	13.3	2.1	-
35–64	3530	6011	45	7.5	1.3	-
≥65	613	954	8	8.4	1.3	-
Any medical risk factor	17,101	30,734	27	.9	-	-
Positive, total	1376	2074	18	8.7	1.3	-
Low positive	478	746	2	2.3	0.4	-
Medium positive	418	611	2	3.3	0.5	-
High positive	480	707	14	19.8	2.9	-
Negative	15,176	27,808	7	0.3	-	99.9
Inconclusive	549	852	2	2.3	-	-
Foreign-born, total	19,418	34,010	183	5.4	[0.9]	[99.1]
Positive, total	8306	13,480	164	12.2	2.0	-
Low positive	1474	2497	13	5.2	0.9	-
Medium positive	2176	3553	36	10.1	1.7	-
High positive	4656	7430	115	15.5	2.5	-
Negative	10,871	20,147	14	.7	-	99.9
Inconclusive	241	382	5	13.1	-	-
Norwegian-born, total	25,457	46,262	22	.5	[0.09]	[99.9]
Positive, total	1572	2322	12	5.2	0.8	-
Low positive	692	1096	1	.9	0.1	-
Medium positive	494	706	2	2.8	0.4	-
High positive	386	520	9	17.3	2.3	-
Negative	23,257	42,917	10	.2	-	99.9
Inconclusive	628	1022	-	-	-	-
≥2 years after QFT® (until TB, LTBI treatment, death, emigration, or study end)						
Total study population	39,942	88,520	52	0.6	[0.1]	[99.9]
Positive, total	7132	17,041	43	2,5	0.6	-
Low positive	1679	4095	3	0.7	0.2	-
Medium positive	1910	4703	8	1.7	0.4	-
High positive	3543	8243	32	3.9	0.9	-
Negative	32,124	70,123	9	0.1	-	99,9
Inconclusive	686	1356	-	-	-	-

[a]Low positive, IFN-γ 0.35 to <1.0 ; medium positive, IFN-γ 1.0 to <4.0; and high positive, IFN-γ ≥ 4.0 IU/ml.
[c]For a hypothetical test in which all individuals tested positive [for hPPV] or all tested negative [for hNPV].
TB, incident tuberculosis; Years, sum of person-years follow-up; IFN, interferon; IR, incidence rate (/1000 person-years); PPV, positive predictive value; hPPV, hypothetical positive predictive value; NPV, negative predictive value; hNPV, hypothetical negative predictive value

Number needed to treat (NNT)

Four foreign-born adults developed tuberculosis disease after LTBI treatment (10 months, 2.5 years, 4.9 years and 5.1 years after LTBI treatment). The NNT decreased substantially with higher IFN-γ categories (table 4). The NNT were overall higher in the Norwegian-born compared with the foreign-born except in the high IFN-γ category.

Table 4 The average number of LTBI treatments needed to prevent one incident TB by result of QuantiFERON® TB Gold (n=44,875)

	No LTBI treatment		LTBI treatment		NNT [95% CI]
	n	TB	n	TB	
Total study population	42,433	257	2442	4	
Positive, total	7747	219	2131	4	38 [35-41]
Low positive	1809	17	357	0	106 [102-112]
Medium positive	2084	46	586	3	59 [44-90]
High positive	3854	156	1188	1	25 [24-27]
Negative	33,835	33	293	0	1025 [1014-1036]
Inconclusive	851	5	18	0	170 [159-182]
Foreign-born, total					
Positive, total	6580	164	1726	4	34 [32-38]
Low positive	1252	13	222	0	78 [74-83]
Medium positive	1735	36	441	3	54 [38-92]
High positive	3593	115	1063	1	25 [24-27]
Negative	10760	14	111	0	598 [587-609]
Inconclusive	229	5	12	0	46 [41-53]
Norwegian-born, total					
Positive, total	1167	12	405	0	90 [85-95]
Low positive	557	1	135	0	557 [514-607]
Medium positive	349	2	145	0	175 [158-195]
High positive	261	9	125	0	26 [23-30]
Negative	23075	10	182	0	1538 [1519-1558]
Inconclusive	622	0	6	0	-

[a]Low positive, IFN-γ 0.35 to <1.0; medium positive, IFN-γ 1.0 to <4.0; high positive, IFN-γ ≥ 4.0 IU/ml.
TB, tuberculosis; LTBI, latent tuberculosis infection; IFN, interferon; NNT, numbers needed to treat; CI, confidence interval

Competing risks

Thirteen percent (*n* = 5745) of individuals [18% (*n* = 3465) foreign-born, 9% (*n* = 2280) Norwegian-born] were censored due to non-event occurrence during the study period. Among censored foreign-born individuals, 1849 (54%) received LTBI treatment, 283 (8%) died, and 1333 (38%) emigrated. Among censored Norwegian-born individuals, 593 (26%) received LTBI treatment, 1578 (69%) died, and 109 (5%) emigrated.

DISCUSSION

Using a large population-based prospective cohort of individuals with QFT® results linked to demographic and health registry data, we explored the prognostic value of the QFT® in a low-TB-incidence country. Hazard ratios for incident TB increased with IFN-γ levels until a plateau of 1.0–4.0 IU/ml, above which further increase was not associated with additional prognostic information. Consistently, in all analyses and across subgroups, individuals in higher IFN-γ categories were more likely than those with low positive levels to develop incident TB. This observation was supported by

the results of sensitivity analyses based on a 6-month cut-off for incident TB and when restricting the outcome to TB confirmed by culture. Our main findings were clinically significant (hazard ratios greater than 8) and are therefore not statistical artefacts. The high number of individuals with a final inconclusive QFT® was surprising and may reflect that clinicians may have decided to base their follow up on the result of a TST-result, rather than an inconclusive QFT®.

Associations between IFN-γ levels and incident TB

Some authors,[9 13-16] but not others,[15] have reported increased risks of subsequent TB with higher mean IFN-γ levels. Substantial overlap in IFN-γ levels between individuals with incident TB and those who remain healthy yields low prognostic accuracy. In contrast to the TST, for which cut-off levels differ among risk groups, a single cut-off level is used to define QFT® positivity. The manufacturer's cut-off level at 0.35 IU/ml was established to maximize sensitivity and specificity and was based on a relatively small study including 118 patients with culture confirmed TB and 216 healthy controls.[17] Interestingly, the same study group later suggested to lower the cut-off for immunosuppressed groups and increase it for low-risk immunocompetent individuals.[18]

A considerable number of reversions from marginal QFT® positivity to negativity have been reported.[19] In a systematic review examining the reproducibility of IGRA findings based on second samples obtained from individuals within 4 weeks after first sample collection, 57% of subjects (primarily health care workers undergoing screening) with baseline IFN-γ levels of 0.35–0.8 IU/ml showed reversion.[19] In the current study, almost one in four individuals with QFT® positivity had IFN-γ levels < 1.0 IU/ml. This finding adds fuel to the debate on whether low positive results should be reported as borderline ('grey zone') to inform clinicians about the lower confidence in the test result. Also, discussions are ongoing regarding whether 'retesting zones' should be recommended, or if cut-offs should differ based on background risk. [20 21] Furthermore, the QFT® is unlikely to distinguish infections that have cleared. [22] Our data suggest that a medium or high positive result adds confidence to an LTBI diagnosis relative to a low positive level in an immunocompetent individual, comparable to results reported for the TST. Interestingly, consistent with our QFT® results, a similar increased risk of incident TB has been observed with higher TST indurations.[23 24] In a large population-based study in Canada, higher TB IRs were observed with TST indurations $\geq$ 15mm compared to TST 10-14mm or 5-9 mm for both close and casual contacts.[23 24]

However, as both tests are based on immune response, the results must be interpreted with caution in immunosuppressed individuals – who are more likely to test negative or low positive, despite being at greater risk of incident TB.[2]

The prognostic value of the QFT®

Our findings confirm previous reports of the low prognostic value of QFT® for subsequent TB in a low-incidence setting.[2 25-27] The TB IR of 11.1/1000 person-years in QFT®-positive individuals in the current study is in the lower range of IRs reported in previous meta-analyses (4–48[27] and 3.7–84.5[2]/1000 person-years in IGRA-positive individuals), and higher than in a recent Danish population-based study (3.8/1000 person-years).[9] The NNT in QFT® positivity was similar to a European study on TB contacts,[15] but lower than reported in the Danish study.[9 28]

The overall PPV of 2.2% from the full study-period was comparable to the 1.9%[15]and 1.32%[9] reported from other low-incidence countries, and lower than the pooled PPV of 2.7% reported in a meta-analysis.[29] Direct comparison is difficult, given differences in study designs, populations, and follow-up periods. The highest PPVs have been obtained in studies of TB contacts (2.4–28.6%)[13 30] and immunocompromised individuals (7–8%).[31] Reported PPVs are probably underestimated, since follow-up is restricted and incident TB may occur over a lifetime. The NNT may be overestimated for

the same reason. The large number of test positive individuals not starting LTBI treatment is of concern. A previous Norwegian study found poor information flow of screening results from immigrant arrival screening which may contribute to the findings.[32] Norwegian guidelines recommend treatment in high-risk QFT® positive individuals and conditionally recommends LTBI treatment in healthy low-risk individuals. Individuals who do not start treatment should be informed about LTBI and common TB symptoms for early case-detection and should preferably be scheduled for follow-up visits. This study is one of several ongoing projects in Norway aiming to address this concern.

Predictive values depend greatly on the prevalence of the condition in the population to which they are applied. In our study, the QFT® added very little predictive information to that provided by the hypothetical tests, due to the low TB prevalence compared to the large number of tests. A large proportion of the Norwegian-born group was tested prior to DMARD treatment. In this group, the pre-test probability of LTBI is low and positive results likely represents remote infection. As the overall lifetime risk of progression from LTBI to TB is low (<5% in healthy populations), high PPVs are very difficult to obtain. [2 27] Inversely, NPVs are high in low-risk populations. Thus, the targeting of groups with high TB risk is essential to improve PPVs. A new-generation QFT® (QuantiFERON® TB Gold Plus) has recently been developed, and has the capacity to detect a larger proportion of CD8+ T cell responses.[33 34] Although this new test has been assessed in several studies,[35-38] no strong evidence of superior performance compared with the QFT® has been produced to date.[39] The prognostic value of the QFT Plus needs to be studied prospectively.

The risk of incident TB following medical risk factors

Although a large proportion (38%) of individuals in this study population had at least one medical risk factor, this characteristic was not associated significantly with incident TB when included in the analysis as a compound risk factor. Possible explanations are: (i) the high sensitivity of our definitions of underlying risk, which may have diluted the effects of the most severe immunosuppressive conditions; (ii) the difficulty of estimating levels of immunosuppression from register data; and (iii) the likely moderate to low immunosuppressive effects of most risk factors. To this point, the majority (94%) of HIV-positive individuals were receiving antiretroviral treatment. Factors currently used to identify risk of progression to disease have, with few exceptions, relatively weak impacts and are insufficient to be drivers of the transition toward disease.[40 41]

Strengths and limitations

The main strengths of our study are the population-based prospective design, large sample with nation-wide coverage, long follow-up time, and standardised information with a high degree of completeness in the cohort. Furthermore, we applied comprehensive statistics to correct for competing risks for the main outcome, time-varying factors, and repeated QFT® testing.

The main limitations include the ineligibility of many recent immigrants (primarily asylum seekers), who had not yet been provided with valid identification numbers, preventing data linkage. Their risk profiles may differ due to the emigration process. Representativeness is crucial for prevalence estimates, but may be less essential for association estimates.[42] Therefore, we believe that we may cautiously generalise the associations found in this study to the broader population.

Information on the indication for the QFT® test was not available. This information would be useful for improved targeting of risk groups. We do not know the extent to which the QFT® was performed subsequently to positive TST findings, as per the guidelines during the study period. However, we believe that the guidelines for screening in primary health care were routinely followed. The percentage of positive QFT® results among those tested after TST positivity will be higher than in studies in which the QFT® was the initial test. However, associations between QFT®

results and incident TB may be less affected due to the comparable sensitivity (80%) of the two tests.[2] The large number of negative QFT® results may reflect the superior specificity of the QFT® compared with the TST (94% vs. 88.7%).[2 43]

The broad classification of immunosuppressive risk may have diluted the effects of the most severe risk factors and overestimated others. Furthermore, the probability of immigrants having health information captured in a national registry may differ based on the time spent in Norway. Thus, under- and overestimation of the prevalence of underlying risk factors in this study is possible.

Public health implications

The overall low ability of the QFT® to predict incident TB is of concern.[15 28] Whereas QFT® negativity provides confidence of low TB risk, the interpretation of a low positive result is less straightforward. Our results, which consistently showed greater risk of incident TB in higher positive IFN-γ categories, may aid the targeting of individuals for preventive treatment. Targeting individuals in higher IFN-γ categories will significantly reduce the NNT to prevent one incident TB. This raises the question of whether separate cut-off values based on background risk could be useful. Furthermore since the majority of incident TB events occurred the first two years after QFT® testing, timely follow-up of test results is necessary to prevent incident TB.[15 28]

We fully support ongoing collaborative initiatives to develop novel tests that may better distinguish different phases in the LTBI spectrum and improve the prognostic value of LTBI diagnosis. Meanwhile, TB control programmes need to target individuals considered to be at greatest risk of progression to TB.

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Data from Norwegian Patient Register at the Norwegian Directorate of Health, Norwegian Prescription Database and Norwegian Surveillance System for Infectious Diseases at the Norwegian Institute of Public Health, and Statistics Norway were used for this publication. The interpretation and reporting of these data are the sole responsibility of the authors, and no endorsement by the registries is intended or should be inferred.

Ethical considerations

The Norwegian Data Protection Authority (14/01138) and the Regional Committee for Medical Research Ethics (2014/1202) approved the study. An external partner de-identified data before the researchers were given access to it.

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Competing interests

None declared.

Authors' contributions

All authors were involved in planning of the study. BAW initiated the study. BAW and RW were responsible for data-management, statistical analysis and for drafting the manuscript, HS, DHS, EKH, NH, GSS, JEA and AMBK provided and cleaned QFT® data for the study, HSB and ABB were responsible for classification of medical risk factors, BAW, RW, ABB, HSB, HS, DHS ATM, FO and

AMBK were involved in the writing process. All authors have reviewed and approved the final version of the manuscript.

Figure legends

Figure 1 Study population flow chart

Solid lines: population included in data-linkage, Stippled lines: population excluded from the IFN-γ study, Shaded box: population included in the IFN-γ study based on level of IFN-γ IU/ml

Figure 2ab Hazard ratios for incident tuberculosis by IFN-γ level compared with the reference level of 0.35 IU/ml (*n* = 41,533 individuals) for two models with different knot-values

Only results with IFN-γ < 10.0 IU/ml were included in the models.
Grey shaded areas represent negative (<0.35 IU/ml), and low positive (> 0.35 to < 0.7 IU/ml, and >0.7 to <1.0 IU/ml) IFN-γ levels
The model with fewer knots had an AIC of 3128 and the model with more knots had an AIC of 3103, suggesting that the model with more knots fit the data better.

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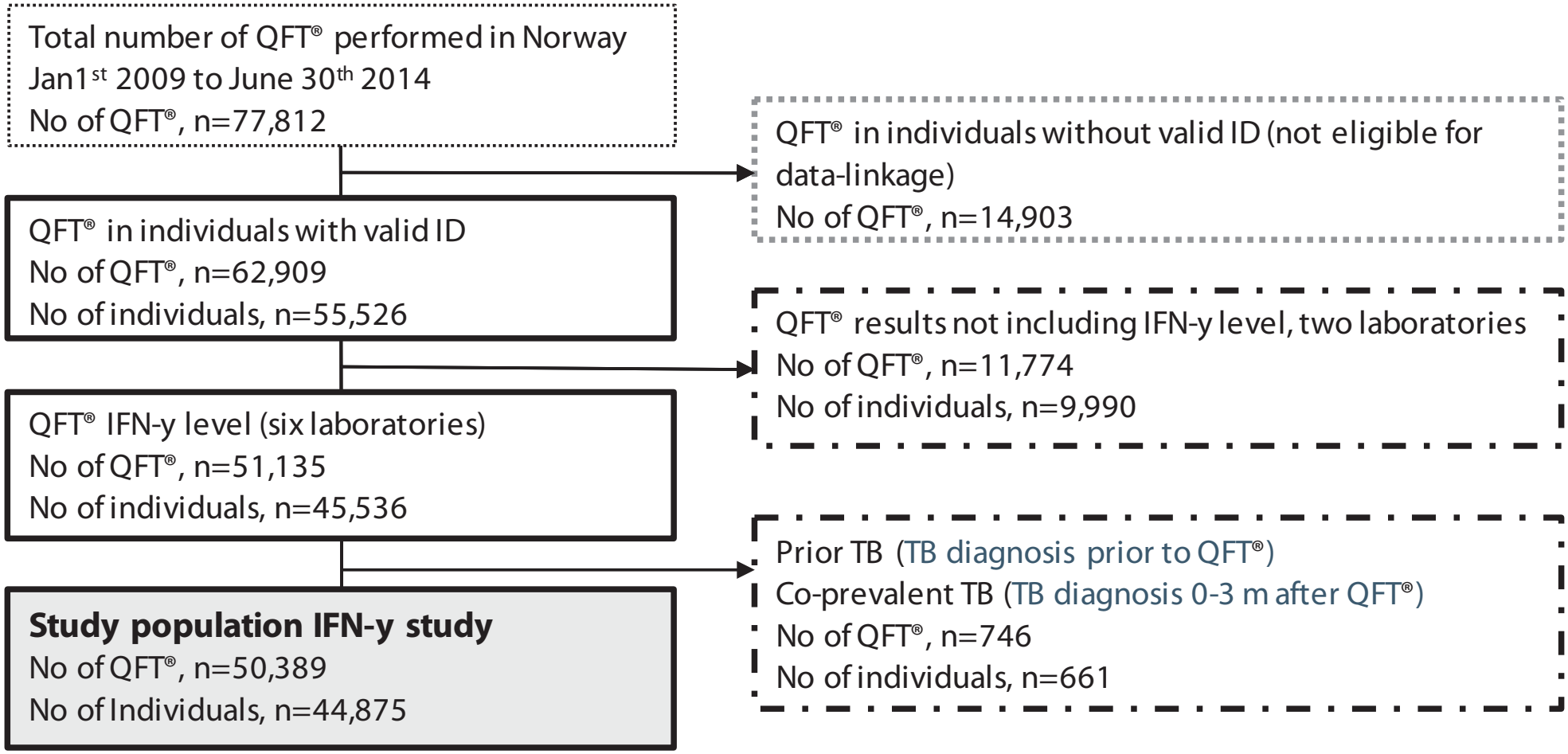
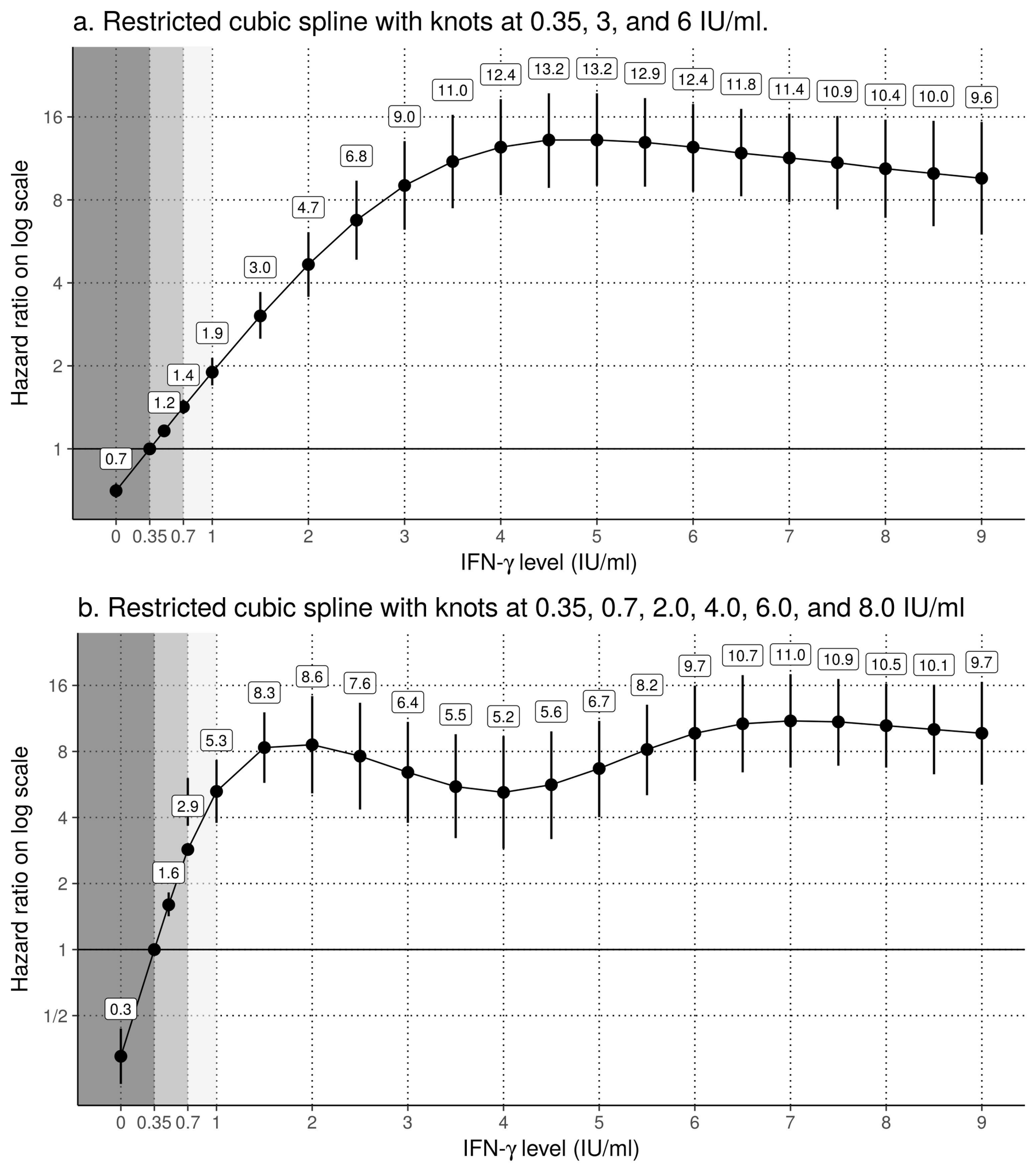

Total number of QFT® performed in Norway
Jan1st 2009 to June 30th 2014
No of QFT®, n=77,812
QFT® in individuals without valid ID (not eligible for data-linkage)
No of QFT®, n=14,903
QFT® in individuals with valid ID
No of QFT®, n=62,909
No of individuals, n=55,526
QFT® results not including IFN-y level, two laboratories
No of QFT®, n=11,774
No of individuals, n=9,990
QFT® IFN-y level (six laboratories)
No of QFT®, n=51,135
No of individuals, n=45,536
Prior TB (TB diagnosis prior to QFT®)
Co-prevalent TB (TB diagnosis 0-3 m after QFT®)
No of QFT®, n=746
No of individuals, n=661
Study population IFN-y study
No of QFT®, n=50,389
No of Individuals, n=44,875


a. Restricted cubic spline with knots at 0.35, 3, and 6 IU/ml.
Hazard ratio on log scale
16
8
4
2
1
0.7
1.2
1.4
1.9
3.0
4.7
6.8
9.0
11.0
12.4
13.2
13.2
12.9
12.4
11.8
11.4
10.9
10.4
10.0
9.6
0 0.35 0.7 1 2 3 4 5 6 7 8 9
IFN-γ level (IU/ml)
b. Restricted cubic spline with knots at 0.35, 0.7, 2.0, 4.0, 6.0, and 8.0 IU/ml
Hazard ratio on log scale
16
8
4
2
1
1/2
0.3
1.6
2.9
5.3
8.3
8.6
7.6
6.4
5.5
5.2
5.6
6.7
8.2
9.7
10.7
11.0
10.9
10.5
10.1
9.7
0 0.35 0.7 1 2 3 4 5 6 7 8 9
IFN-γ level (IU/ml)

Appendix 1 Classification of immunosuppressive risk factors for tuberculosis disease

ATC codes classified according to: WHO Collaborating Centre for Drug Statistics Methodology, ATC classification index with DDDs 2016. Oslo 2015.
ICD-10 codes classified according to: Directorate of e-health Norway, International Classification of Diseases 10th revision. 2017

Marker of risk			ICD-10/[NCMP]-codes [I]	AND/OR	ATC-codes [II]
Underlying disease, any			Summary of lines 1-15		Summary of lines 1-15
1	HIV infection		B20-B24, O987, R75, Z21	OR	J05AR
2	Diabetes		E10-E14 O24	OR AND	A10 A10
3	Silicosis		J65		
4	Chronic renal disease with or without haemodialysis		N01, N03, N04, N11, N18, [KAGD40, Z992*, JAGD30, JAGD31, JAGD50]	OR	V03AE02, V03AE03
5	Malignant neoplasms, any		C00-C97		
6	Solid organ transplant		T86.0-T86.4, T86.8, T86.9, Z94		
	Diseases relevant for DMARDs treatment, any [III]		Summary of lines 7-12		
7		Inflammatory polyarthropathies	M05-M13		
8		Systemic connective tissue disorders	M30-M35		
9		Spondylopathies	M45-M46		
10		Papulosquamous disorders	L40-L41		
11		Non-infective enteritis and colitis grouped	K50-K51		
12		Multiple sclerosis	G35		
13	Malnutrition		E40-E46		
14	Dependence syndrome, alcohol		F10.2	OR	N07BB
15	Dependence syndrome, opioids		F11.2	OR	N07BC01/02, N07BC51
Iatrogenic immunosuppression, any			Summary of lines 16-22	OR	Summary of lines 16-22
16	Antineoplastic agents		[L01XC02]	OR	L01
17	Selective immunosuppressants		[L04AA24]	OR	L04AA
18	TNF-alpha inhibitors		[L04AB01/02/04/05/06]	OR	L04AB
19	Interleukin inhibitors		[L04AC03/05/07]	OR	L04AC
20	Calcineurin inhibitors				L04AD
21	Other immunosuppressants				L04AX
22	Long term steroid treatments				H02AB DDD > 15mg > 1 month

[I] Hospital discharge data from the Norwegian Patient Registry (NPR), ICD-10: International Classification of Diseases 10th revision, NCMP: the Norwegian Classification of Medical Procedures. Underlying diseases included registrations prior to or at the time of administration of the QuantiFERON®TB-Gold

[II] Outpatient prescriptions data from the Norwegian Prescription Database (NORPD): ATC: Anatomical Therapeutic Chemical Classification System. Iatrogenic immunosuppression included drugs if there was at least one prescription within the last six months prior to QuantiFERON®TB-Gold

[III] DMARDs, Disease Modifying Anti-Rheumatic Drugs

Appendix 2, Detailed overview of statistical analyses of the main exposure and outcome

The main exposure was interferon-gamma (IFN-γ) levels in IU/ml reported as recommended by the manufacturer. Since risk of tuberculosis may change over time, and some individuals have more than one QFT®-test, we applied time-dependent Cox regression model to examine associations between the main outcome and the main exposure. This involved constructing a row of data for each QFT®, from the start of the interval (date of sampling) until the end of the interval (event, censoring or date of sampling for a subsequent test). Covariate values are those that apply over that interval. Using time-varying explanatory variables is more robust than selecting exposures from a single time point as it utilizes all available data.

Splines and categorization of IFN-γ levels

We had a priori information that the association between incident TB and IFN-γ levels in IU/ml was non-linear. Three laboratories only reported continuous IFN-γ levels until 10 IU/ml, and then reported "≥ 10 IU/ml" for the remaining. We therefore decided to model the continuous data using restricted cubic splines, which would give us insight into appropriate categorizations of the data and allowing usage of all the available results. Only tests with IFN-γ levels below 9.99 IU/ml were included in the spline models. We ran two regressions (including origin, age and identified medical risk factors as adjustment variables), one with knots at 0.35, 3, and 6 IU/ml, and the other with knots at 0.35, 0.7, 2.0, 4.0, 6.0 and 8.0 IU/ml. The lowest knot values (0.35, 0.7 and 1.00 IU/ml) were selected based on clinical interest, and the remaining on equal spacing. Both regressions supported the categorization of IFN-γ levels as negative at < 0.35 (according to manufacturer's cut-off), low positive at 0.35 to <1.0 IU/ml, medium positive at 1.0 to < 4.0 IU/ml, and high positive at ≥ 4.0 IU/ml. We used these categories in all further analyses.

Effect modification and interaction terms

To investigate if the association between IFN-γ levels IU/ml and incident TB disease was modified by origin, age or identified medical risk factor, we ran the following models:

(i) baseline model: incident TB = (categorized IFN-γ levels) + (age) + (origin) + (medical risk factor),

(ii) modified by origin model: incident TB = (categorized IFN-γ levels) + (age) + (origin) + (medical risk factor) + (categorized IFN-γ levels)*(origin), and

(iii) modified by age model: incident TB = (categorized IFN-γ levels) + (age) + (origin) + (medical risk factor) + (categorized IFN-γ levels)*(age).

(iv) modified by medical risk factors model: incident TB = (categorized IFN-γ levels) + (age) + (origin) + (medical risk factor) + (categorized IFN-γ levels)*(medical risk factor).

We then performed likelihood ratio tests comparing the various models to the "baseline model". We found no statistically significant effect of age, origin or identified medical risk factors on the IFN-γ levels (IU/ml) and these co-variates were included in the model.

Numbers needed to treat (NNT)

Number needed to treat for latent tuberculosis infection to prevent one case with incident tuberculosis disease was calculated by estimating the difference in risk of incident TB among individuals who did not and those who did receive LTBI treatment. NNT=1/(incident TB/number of individuals not receiving LTBI treatment –incident TB/individuals receiving LTBI treatment)

Appendix 3, Sensitivity analysis – definition of incident tuberculosis disease

In this sensitivity analysis, we defined a case as incident tuberculosis (TB) if date of sample collection for TB diagnosis was ***more than six months* after the QFT® administration**, as compared to *more than three months* after QFT® administration in the main analyses

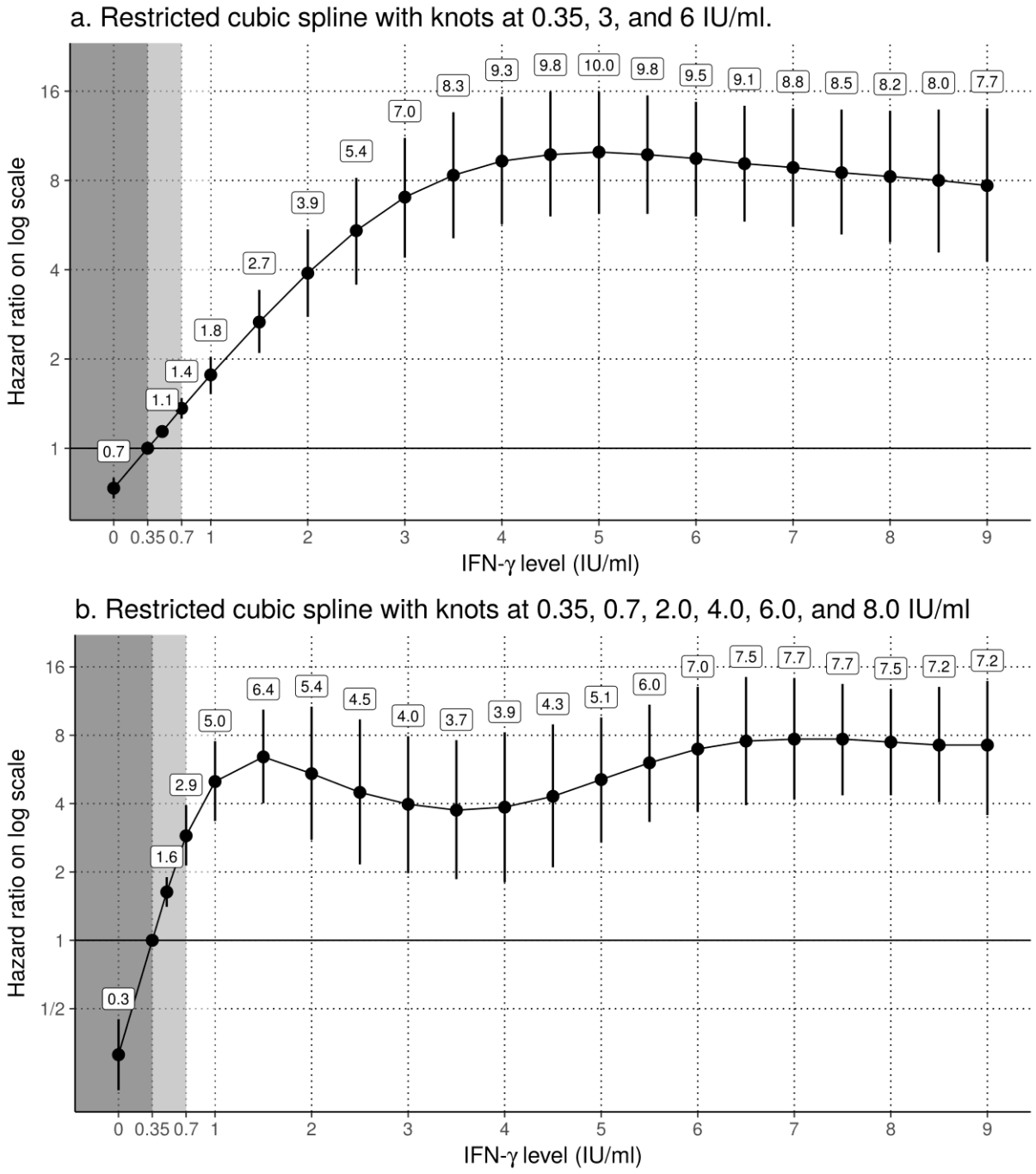
Hazard ratios for incident tuberculosis disease (n=170) by IFN-γ level, origin, age and medical risk-factors, n=43923, by time-dependent Cox regression*

Covariates	TB events	HZ	*p*	95% CI
IFN-γ level IU/ml [a]				
IFN-γ < 0.35	20	0.15	< 0.001	0.07-0.36
IFN-γ >=0.35 to < 1.0	9	1 (ref)		
IFN-γ >= 1.0 to < 4.0	30	2.28	0.030	1.08-4.82
IFN-γ >= 4.0	111	4.30	< 0.001	2.16-8.46
Origin				
Foreign-born	156	1 (ref)		
Norwegian-born	14	0.40	0.004	0.21-0.74
Age-group				
Age ≥ 35 yrs	117	1 (ref)		
Age < 35 yrs	53	1.65	0.003	1.19-2.30
Any medical risk factor [b]				
No risk factors	150	1 (ref)		
At least one risk factor	20	1.42	0.193	0.84-2.39

TB events, TB diagnosed more than 6 months after the QFT® administration; Yrs, sum of person years follow-time after QFT®; HZ, hazard ratio

[b] *Information about medical risk factors is based on ICD10/NCMP codes from Norwegian Patient Registry and ATC-codes from Norwegian Prescription Registry.*

Appendix 4 Hazard ratios for incident **culture confirmed** tuberculosis (n=150) by IFN-γ level compared with the reference level of 0.35 IU/ml (*n* = 41,431 individuals).



Only results with IFN-γ < 10.0 IU/ml were included in the models. Individuals with TB not confirmed by culture (=102) were excluded from the analyses.

Grey shaded areas represent negative (<0.35 IU/ml), and low positive (> 0.35 to < 0.7 IU/ml, and >0.7 to <1.0 IU/ml) IFN-γ level