PAPER 1

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Low prevalence of positive interferon-gamma tests in HIV-positive long-term immigrants in Norway

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SUMMARY

OBJECTIVE: To determine the prevalence and predictors of positive interferon-gamma release assays (IGRAs) and tuberculin skin tests (TSTs) in human immunodeficiency virus (HIV) infected patients in Norway, a low tuberculosis (TB) endemic country.

DESIGN: Multicentre cross-sectional study of 298 HIV patients tested with QuantiFERON®-TB Gold In-Tube (QFT-GIT), T-SPOT®.*TB* (T-SPOT) and TST.

RESULTS: A total of 77/298 (26%) QFT-GIT, 29/117 (25%) T-SPOT and 52/217 (24%) TSTs (≥5 mm) were positive. The median CD4 count was 427 cells/μl. Three QFT-GIT results but no T-SPOT results were indeterminate. Of 52 TST-positive patients, 34 (65%) were QFT-GIT-positive (median interferon-gamma [IFN-γ] 4.38 international units [IU]/ml), compared to 16% of the TST-negative patients (median INF-γ 0.81 IU/ml, $P < 0.001$). Origin from a TB-endemic country, previous active TB and TB exposure were associated with a positive QFT-GIT ($P \leq 0.01$). Patients from TB-endemic countries living in Norway for ≥10 years had lower odds of a positive QFT-GIT (12%; OR 0.17, 95%CI 0.06–0.53, $P = 0.002$) than patients with 0–3 years' residence (49%).

CONCLUSION: The prevalence of positive IGRAs in HIV-infected patients was high in this low TB endemic setting. Lower QFT-GIT positivity in long-term residents from TB-endemic countries may reflect a waning of TB-specific immune responses.

KEY WORDS: tuberculosis; IGRA; QuantiFERON; tuberculin skin testing; low-endemic country

TUBERCULOSIS (TB) is the most common opportunistic infection in human immunodeficiency virus type I (HIV) infected patients, while HIV in turn increases the risk of re-activation of latent tuberculous infection (LTBI). Interferon-gamma release assays (IGRAs) are recommended as a supplement to or instead of the tuberculin skin test (TST) in the diagnosis of LTBI in high-income, low TB endemic settings.[1–4] A recent systematic review and meta-analysis reported a sensitivity of 69% for T-SPOT®.*TB* (T-SPOT) based on one study and of 59% for QuantiFERON®-TB Gold In-Tube (QFT-GIT) based on five studies of HIV-infected patients living in low TB endemic countries.[5] However, few studies have compared the various tests in the diagnosis of LTBI in HIV-infected patients in this setting.

The incidence rate of TB in Norway is 7 per 100 000 population and the Norwegian Surveillance System for Communicable Diseases (MSIS) reports approximately 350 new TB cases annually, of which 80–90% occur in immigrants.[6] Similarly, of the approximately 300 cases of newly diagnosed HIV infections registered each year, 60–70% occur in immigrants.[7] However, no national surveillance data on TB-HIV coinfection are available.

In this multicentre study of HIV-positive patients from seven hospitals in Norway, we aimed to evaluate the performance of IGRAs and TST in an HIV-positive population and determine the prevalence and risk factors associated with positive tests in this low TB endemic setting.

STUDY POPULATION AND METHODS

HIV-positive individuals aged >18 years were recruited between January 2009 and October 2010 from out-patient clinics at seven hospitals in Norway. Demographics, background information on HIV infection

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and risk factors for TB were obtained by self-report and from medical records. Blood samples for CD4 counts and HIV-RNA were drawn and patients underwent chest X-ray. Induced sputum samples were obtained for acid-fast staining and culture. TST was performed after the IGRA test to avoid the possibility of boosting, and read after 72 h (0.1 ml tuberculin purified protein derivative RT23 2 TU, Statens Serum Institute, Copenhagen, Denmark).[8] A cut-off of ⩾5 mm induration was considered a positive test according to Centers for Disease Control and Prevention guidelines for HIV-infected persons.[9] Clinical examinations were performed among all participants by the attending clinician at the respective clinics, and active TB was ruled out. The attending clinician decided indications for prophylactic treatment consisting of either 6 months of isoniazid (INH) or 3 months of rifampicin and INH per national guidelines.[2] The entire cohort was followed for a period of 24 months.

The study was approved by the Regional Committee for Medical and Health Research Ethics (REK-Vest, REK-Nord) and by the Norwegian Social Science Data Services (NSD), Bergen, Norway. Written informed consent was obtained from each participant.

Interferon-gamma release assays

The QFT-GIT assay was performed and interpreted according to the manufacturers' instructions (Cellestis Ltd, Qiagen, Chadstone, VIC, Australia). Interferon-gamma (IFN-γ) ⩾ 10 international units (IU)/ml were recorded as 10 IU/ml due to the uncertainty of tests results above that level. IFN-γ < 0.35 IU/ml (negative test) was recorded as 0.34 IU/ml. T-SPOT.*TB*® was performed and interpreted per the manufacturers' instructions (Oxford Immunotec Ltd, Abingdon, UK) in parallel to QFT-GIT among participants attending one of the seven hospitals. Positive results were defined as >7 spot forming units (sfu), negative results as <5 sfu and borderline values were set at 5, 6 or 7 sfu, per the manufacturers' recommendations.

Statistical analysis

STATA 12 software (Stata Corp, College Station, TX, USA) was used for statistical analysis. Results are given as medians and ranges. Differences between groups were assessed using the χ^2 test for categorical variables and the Kruskal-Wallis rank-sum test for continuous variables. Univariate and multivariate odds ratios (ORs) were obtained using logistic regression models. Concordance between both IGRAs and between TST and IGRAs were determined by calculating Cohen's kappa coefficient (κ), where κ < 0.4 represented poor agreement, 0.4–0.6 fair to moderate agreement, 0.6–0.8 good agreement and >0.8 excellent agreement.

RESULTS

Table 1 summarises the baseline characteristics and corresponding IGRA results of the 298 HIV-positive patients recruited for the study. The majority of the

Table 1 Baseline characteristics of study participants and IGRA results†

Characteristic	All (*n* = 298) median [range] or *n* (%)	QFT-GIT+ (*n* = 77) median [range] or *n* (%)	QFT-GIT- (*n* = 218) median [range] or *n* (%)	*P* value*	All (*n* = 117) median [range] or *n* (%)	T-SPOT.*TB*+ (*n* = 29) median [range] or *n* (%)	T-SPOT.*TB*− (*n* = 84) median [range] or *n* (%)	*P* value*
Age, years	40 [19–73]	38 [20–60]	41 [19–73]	0.012	41 [19–73]	39 [23–52]	41.5 [19–73]	0.101
Female	160 (54)	43 (56)	117 (54)	0.742	52 (44)	9 (31)	41 (49)	0.097
Origin from TB-endemic country	215 (72)‡	71 (93)	142 (65)	<0.001	84 (72)¶	29 (100)	52 (62)	<0.001
Years of residence in Norway#	6 [0–38]	5 [0–17]	7 [0–38]	0.002	6 [0–20]	4 [0–16]	7 [0–20]	0.128
Previous AIDS diagnosis	145 (49)	34 (45)	110 (51)	0.372	62 (53)	12 (41)	47 (56)	0.176
Years since HIV diagnosis	6 [0–25]	4 [0–22]	6 [0–25]	0.003	5 [0–25]	4 [0–15]	6 [0–25]	0.038
Nadir CD4 count	190 [0–1160]	226 [0–1160]	180 [1–930]	0.003	190 [5–1160]	270 [10–1160]	180 [5–930]	0.049
Enrolment CD4 count	427 [3–1870]	470 [50–1870]	400 [3–1430]	0.127	390 [20–1870]	410 [50–1870]	370 [20–1430]	0.462
ART	199 (67)	41 (54)	155 (71)	0.006	67 (57)	11 (38)	53 (63)	0.018
BCG-vaccinated	229 (79)	52 (69)	174 (82)	0.038	108 (97)	26 (93)	78 (99)	0.106
Contact with TB patient	36 (12)	18 (24)	18 (8)	<0.001	7 (6)	3 (10)	4 (5)	0.282
Visits to TB-endemic country**	43 (15)	3 (60)	39 (52)	0.729	15 (13)	0	15 (18)	—
Previous active TB	32 (11)	16 (21)	15 (7)	0.001	17 (15)	7 (24)	9 (11)	0.074

*Based on Pearson's χ^2 test for categorical variables and Kruskal-Wallis rank-sum test for continuous variables.
†Indeterminate QFT-GIT (*n* = 3) and borderline T-SPOT.*TB* (*n* = 4) results not included.
‡76% African, 16% South-East Asian, 5% Eastern European, remaining 3% from South America, Caribbean and the Middle East.
¶80% African, 17% South-East Asian, remaining 3% from Eastern Europe, Middle East and South America.
#Applies to HIV-patients from TB-endemic countries.
**Applies to HIV patients from non-TB-endemic countries.
IGRA = interferon-gamma release assays; QFT-GIT= QuantiFERON®-TB Gold; + = positive; − = negative; TB = tuberculosis; AIDS = acquired immune-deficiency syndrome; HIV = human immunodeficiency virus; ART = antiretroviral therapy; BCG = bacille Calmette-Guérin.

participants originated from TB-endemic countries (72%) and had resided for a median of 6 years (range 0–38) in Norway. The median CD4 count at enrolment was 427 cells/μl (range 3–1870); only 36 (12%) patients had CD4 counts of ≤200 cells/μl; 67% of the patients were on antiretroviral therapy (ART), 32 (11%) had completed treatment for active TB a minimum of 12 months before enrolment, and four (1%) had previously received TB preventive therapy.

Of 77 QFT-GIT+ patients, 60 (78%) were diagnosed with LTBI and 36 (60%) received prophylactic treatment. None of the treated or untreated patients developed active TB during the observation time of 24 months.

QFT-GIT and T-SPOT responses

Of 298 patients who underwent QFT-GIT testing, 77 (26%) were positive (Table 1), with a median INF-γ level of 2.86 IU/ml (range 0.39–10 IU/ml). There was no significant difference in the frequency of positive QFT-GIT between patients with CD4 count of ≤200 cells/μl and those with CD4 >200 cells/μl (19% vs. 27%, $P = 0.35$). It is of note that only one of the three QFT-GIT-indeterminate patients had CD4 counts of <200 cells/μl. The median nadir CD4 count was higher in the QFT-GIT-positive group than in the QFT-GIT-negative group (226 cells/μl, range 0–1160 vs. 180 cells/μl, range 1–930, $P = 0.003$), whereas CD4 counts at inclusion were comparable. The majority of the patients on ART were QFT-GIT-negative (78% vs. 21%, $P = 0.006$). Patients from TB-endemic countries with a positive QFT-GIT (33%) had resided in Norway for a shorter period than those with QFT-GIT-negative results. Excluding patients with a previous active TB, 49% of those who had moved to Norway recently (0–3 years) had a positive QFT-GIT compared to 12% of those who had lived in the country for ≥10 years ($P = 0.002$).

The T-SPOT assay was performed on blood samples from 117 of the same patients; the 29 (25%) with positive tests all came from a TB-endemic country (Table 1). No significant difference was observed between the T-SPOT-negative and positive groups concerning length of stay in Norway, although a trend similar to that for QFT-GIT was seen. The four patients with borderline T-SPOT had a median CD4 count of 460 cells/μl (range 400–740). There were no indeterminate T-SPOT results.

Comparison between IGRA and TST results in HIV-patients

Parallel TST and QFT-GIT results were available for 217 patients; in 78, (36%) one or both tests were positive. Overall agreement between TST and QFT-GIT was 80% ($\kappa = 0.47$, $P < 0.001$). Corresponding T-SPOT and TST results were available for 74 patients, also with an agreement of 80% (κ 0.52, $P < 0.001$). Overall, 24% had a TST ≥5 mm, of whom

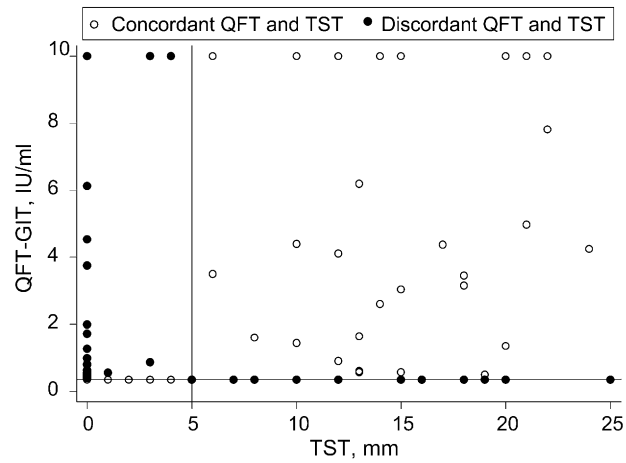

Figure Relationship between QFT-GIT and TST results. Corresponding QFT-GIT (IU/ml) and TST (mm) responses in HIV-positive patients at enrolment ($n = 217$). Cut-off for positive QFT-GIT is ≥0.35 IU/ml (solid line on *y*-axis). Cut-off for positive TST is 5 mm (solid line on *x*-axis). QFT-GIT = QuantiFERON®-TB Gold; TST = tuberculin skin test; IU = international unit; HIV = human immunodeficiency virus.

only 34/52 (65%) were QFT-GIT-positive and 13/18 (72%) were T-SPOT-positive. In contrast, among TST-negative patients, 16% were QFT-GIT-positive and 18% were T-SPOT-positive. The Figure illustrates the spread of TST and QFT-GIT results. There was no significant difference in CD4 count at enrolment between TST+/QFT-GIT+ and TST−/QFT-GIT+ patients (data not shown). Median INF-γ levels were, however, lower in the TST−/QFT-GIT+ group than in the TST+/QFT-GIT+ group (0.81 IU/ml, range 0.39–10 IU/ml vs. 4.38 IU/ml, range 0.5–10 IU/ml, $P < 0.001$). There was no difference in bacille Calmette-Guérin (BCG) status or frequency of previous active TB between the TST+/QFT-GIT− and the TST+/QFT-GIT+ groups; the size of TST was comparable (data not shown).

Parallel T-SPOT and QFT-GIT testing was performed in 117 patients; overall agreement between the IGRA tests, excluding indeterminate QFT-GIT and borderline T-SPOT results, was 91% (κ 0.77, $P < 0.001$; Table 2). Median INF-γ levels were lower in patients with T-SPOT−/QFT-GIT+ results than in those with T-SPOT+/QFT-GIT+ results ($P = 0.017$).

Table 2 Concordance between T-SPOT.*TB* and QFT-GIT*

		QFT-GIT-positive	
T-SPOT.*TB* ($n = 111$)	QFT-GIT-negative	No	IFN-γ value, IU/ml median [range]
Negative	76	6	0.53 [0.39–10]
Positive	4	25	2.00 [0.43–10]

*$\kappa = 0.77$; $P < 0.001$. Borderline T-SPOT.*TB* ($n = 4$) and indeterminate QFT-GIT ($n = 2$) results not included.
QFT-GIT = QuantiFERON®-TB Gold; IFN-γ = interferon-gamma; IU = international unit.

Predictors of positive IGRA and TST in HIV infection

In the univariate analysis, origin from a TB-endemic country, previous active TB, contact with a TB patient and high nadir CD4 counts were all associated with a positive QFT-GIT (Table 3). In contrast, increasing age, years since HIV diagnosis and ART were associated with low odds for a positive QFT-GIT. In the multivariate analysis, only origin from a TB-endemic country, previous active TB and contact with a TB patient remained associated with a positive QFT-GIT as well as with a TST ⩾ 5 mm (Table 4); 50% of the patients previously treated for active TB had a positive QFT-GIT at inclusion. Previous active TB was the only predictor of a positive T-SPOT in multivariate analysis (odds ratio [OR] 5.60, 95% confidence interval [CI] 1.51–20.71, $P = 0.010$; data not shown). The low sample size may explain why statistical significance was not reached for other risk factors. Only previous active TB (OR 17.05, 95%CI 3.79–76.65, $P < 0.001$) and higher CD4 counts at enrolment (OR 1.79, 95%CI 1.01–3.17, $P = 0.048$) were associated with a TST ⩾ 15 mm in multivariate analysis. In contrast, older age was associated with a reduced risk of TST ⩾ 15 mm (OR 0.40, 95%CI 0.19–0.84, $P = 0.017$).

A separate multivariate analysis was performed including only HIV patients from TB-endemic countries to further examine the effect of length of residence in Norway (Table 5). Patients in the four 'length of residence' categories originated from the same, predominantly African, countries. Fewer patients were treated with ART (41% vs. 80%, $P < 0.001$) and the CD4 count was lower (330 cells/μl vs. 409 cells/μl, $P = 0.012$) in the group that had been in the country for 0–3 years compared to the group residing in Norway ⩾10 years. However, in multivariate analysis there was a significantly lower odds for a positive QFT-GIT with ⩾10 years of stay (OR 0.17, 95%CI 0.06–0.53, $P = 0.002$), even when adjusting for ART and CD4 counts at enrolment. The OR for linear trend for years of stay in Norway was 0.87 (95%CI 0.79–0.96, $P = 0.007$). A similar trend was observed for T-SPOT; however, this was not significant, likely due to the low sample size (data not shown). There was no association between length of stay in Norway and TST-positive responses, either at cut-off TST ⩾ 5 mm or TST ⩾ 15 mm.

Table 3 Univariate and multivariate analysis of risk factors associated with a positive QFT-GIT

		Univariate analysis*		Multivariate analysis ($n = 275$)*	
Variable	Total	OR (95%CI)†	*P* value	OR (95%CI)†	*P* value
Sex					
Male	34	1			
Female	43	1.12 (0.67–1.89)	0.660		
Age, years	NA	0.66 (0.49–0.88)	0.004	0.92 (0.60–1.39)	0.685
Origin					
Low TB-endemic country	6	1			
TB-endemic country	71	7.59 (2.94–19.60)	<0.001	6.17 (2.01–18.96)‡	0.001‡
Years since HIV diagnosis	NA	0.65 (0.48–0.90)	0.008	0.75 (0.49–1.14)	0.181
Previous AIDS diagnosis					
No	42	1			
Yes	34	0.79 (0.47–1.34)	0.390		
Nadir CD4, cells/μl	NA	1.53 (1.18–2.00)	0.001	1.44 (0.86–2.42)	0.170
CD4 categories (nadir), cells/μl					
<100	14	1			
100–200	18	1.43 (0.65–3.14)	0.377		
201–349	17	1.28 (0.58–2.82)	0.546		
⩾350	23	3.04 (1.38–6.68)	0.006		
CD4 at enrolment, cells/μl	NA	1.19 (0.92–1.53)	0.183	1.24 (0.81–1.90)	0.316
ART at enrolment					
No	35	1			
Yes	41	0.47 (0.27–0.80)	0.005	0.69 (0.29–1.66)	0.412
Previous active TB					
No	60	1			
Yes	16	3.42 (1.61–7.24)	0.001	4.50 (1.83–11.06)‡	0.001‡
Contact with TB patient					
No	58	1			
Yes	18	3.5 (1.71–7.16)	0.001	4.10 (1.77–9.51)‡	0.001‡
Visits to TB-endemic country§					
No	2	1			
Yes	3	1.35 (0.21–8.54)	0.750		

*Variables with $P < 0.05$ in the univariate analysis and CD4 count at enrolment were included in the multivariate analysis.
†ORs per standard deviation increase for continuous variables (age, years since HIV diagnosis, nadir CD4 count and CD4 count at enrolment).
‡Risk factors with significant ORs in multivariate analysis.
§Applies to HIV-positive patients from non-TB-endemic countries.
QFT-GIT = QuantiFERON®-TB Gold; OR = odds ratio; CI = confidence interval; NA = not available; TB = tuberculosis; HIV = human immunodeficiency virus; AIDS = acquired immune-deficiency syndrome; ART = antiretroviral therapy.

Table 4 Univariate and multivariate analysis of factors associated with a positive TST (⩾5 mm)

		Univariate analysis		Multivariate analysis (n = 199)*	
Variable	Total	OR (95%CI)†	P value	OR (95%CI)†	P value
Sex					
Male	22	1			
Female	30	1.08 (0.58–2.03)	0.806		
Age, years	NA	0.77 (0.55–1.08)	0.125		
Origin					
Low TB-endemic country	3	1			
TB-endemic country	49	6.13 (1.82–20.64)	0.003	5.27 (1.48–18.80)‡	0.010‡
Years since HIV diagnosis	NA	0.91 (0.66–1.26)	0.582		
Previous AIDS diagnosis					
No	35	1			
Yes	17	0.44 (0.23–0.85)	0.014	0.50 (0.17–1.42)	0.190
Nadir CD4, cells/μl	NA	1.64 (1.19–2.25)	0.003	1.14 (0.63–2.09)	0.661
CD4 categories (nadir), cells/μl					
<100	9	1			
100–200	10	0.84 (0.31–2.27)	0.730		
201–349	13	1.14 (0.44–2.95)	0.792		
⩾350	16	3.46 (1.29–9.28)	0.014		
CD4 at enrolment, cells/μl	NA	1.41 (1.04–1.90)	0.028	1.42 (0.90–2.23)	0.127
ART at enrolment					
No	23	1			
Yes	29	0.53 (0.28–1.01)	0.054	0.63 (0.23–1.75)	0.376
Previous active TB					
No	41	1			
Yes	11	3.14 (1.31–7.52)	0.010	6.35 (2.00–20.12)‡	0.002‡
Contact with TB patient					
No	40	1			
Yes	12	3.00 (1.30–6.92)	0.010	4.42 (1.61–12.17)‡	0.004‡
Visits to TB-endemic country§					
No	1	1			
Yes	2	1.14 (0.10–13.62)	0.916		

*Variables with $P < 0.05$ in the univariate analysis and CD4 count at enrolment were included in the multivariate analysis.
†ORs per standard deviation increase for continuous variables (age, years since HIV diagnosis, nadir CD4 count and CD4 count at enrolment).
‡Risk factors with significant ORs in multivariate analysis.
§Applies to HIV-positive patients from non-TB-endemic countries.
TST = tuberculin skin test; OR = odds ratio; CI = confidence interval; NA = not available; TB = tuberculosis; HIV = human immunodeficiency virus; AIDS = acquired immune-deficiency syndrome; ART = antiretroviral therapy.

DISCUSSION

In this study, the first of its kind in Norway, we screened HIV-infected patients for TB infection using IGRA and TST. The prevalence of positive tests was 24–26%, indicating a high degree of LTBI among HIV patients in this cohort consisting predominantly of patients originating from TB-endemic countries. As patients were recruited from seven different clinics spread throughout the country, we believe the study population is representative of Norwegian clinics having a large number of foreign-born HIV-positive patients. We assume that the prevalence of IGRA positivity would be lower in clinics with more ethnic Norwegian HIV patients.

The prevalence of QFT-GIT positivity among both HIV patients in general (26% vs. 4.6%) and HIV patients from TB-endemic countries (33% vs. 16%) is higher in our study than in a Danish study.[10] However, a German study of a comparable low-endemic setting reported a prevalence of 18.9% QFT-GIT positivity and 24% T-SPOT positivity among HIV patients.[11] Furthermore, Kall et al. found a positive T-SPOT prevalence of 10% among patients attending a HIV clinic in the United Kingdom.[12] The high prevalence of LTBI among HIV patients suggests that there should be greater awareness among clinicians about TB screening even in low TB-endemic countries.

Origin from a TB-endemic country, previous active TB and contact with a TB patient were risk factors for IGRA and TST positivity, consistent with findings in other studies.[10,13] However, in our study only half of the patients previously treated for active TB had a positive QFT-GIT. Nadir CD4 count was higher in QFT-GIT-positive patients than in QFT-GIT-negative patients, whereas CD4 count at enrolment was comparable. However, in the multivariate analysis neither of these parameters affected IGRA results. Interestingly, our study revealed that patients from TB-endemic countries who had lived in Norway for ⩾10 years had a significantly lower risk of a positive QFT-GIT than those with shorter stay (12% vs. 49%). None of these patients had previously received preventive anti-tuberculosis treatment, and there were no differences in reported TB exposure or country of origin between the 'length of residence' quartiles.

Table 5 Univariate and multivariate analysis of risk factors associated with a positive QFT-GIT in HIV patients from TB-endemic countries

		Univariate analysis*		Multivariate analysis (n =196)*	
Variable	Total	OR (95%CI)†	P value	OR (95%CI)†	P value
Sex					
Male	30	1			
Female	41	0.66 (0.37–1.19)	0.168		
Age, years	NA	1.06 (0.72–1.55)	0.761	1.36 (0.81–2.26)	0.243
Years since HIV diagnosis	NA	0.57 (0.37–0.86)	0.007		
Previous AIDS diagnosis					
No	42	1			
Yes	29	0.59 (0.33–1.05)	0.074		
Nadir CD4, cells/μl	NA	2.02 (1.43–2.85)	<0.001	1.88 (0.99–3.56)	0.054
CD4 categories (nadir), cells/μl					
<100	12	1			
100–200	15	1.58 (0.65–3.82)	0.309		
201–349	17	1.45 (0.62–3.40)	0.393		
⩾350	23	5.89 (2.34–14.81)	<0.001		
CD4 at enrolment, cells/μl	NA	1.39 (1.03–1.88)	0.033	1.24 (0.75–2.05)	0.393
ART at enrolment					
No	35	1			
Yes	36	0.37 (0.20–0.67)	0.001	0.69 (0.26–1.81)	0.447
Previous active TB					
No	56	1			
Yes	15	2.49 (1.13–5.50)	0.024	4.42 (1.64–11.96)‡	0.003‡
Contact with TB patient					
No	53	1			
Yes	18	4.12 (1.82–9.28)	0.001	6.00 (2.20–16.34)‡	<0.001‡
Residence in Norway, years					
0–3	28	1		1	
4–6	18	0.62 (0.29–1.35)	0.229	0.39 (0.15–1.06)	0.064
7–9	15	0.44 (0.20–0.96)	0.039	0.37 (0.13–1.04)	0.061
⩾10	10	0.27 (0.11–0.64)	0.003	0.17 (0.06–0.53)‡	0.002‡

*Variables with $P < 0.05$ in the univariate analysis and CD4 count at enrolment were included in the multivariate analysis. Years since HIV diagnosis was not included as it is highly correlated with length of stay in Norway ($r = 0.77$), and was not significant in multivariate analysis (OR 0.99, $P = 0.979$).
†ORs per standard deviation increase for continuous variables (age, years since HIV diagnosis, nadir CD4 count and CD4 count at enrolment).
‡Risk factors with significant ORs in multivariate analysis.
QFT-GIT = QuantiFERON® Gold; HIV = human immunodeficiency virus; TB = tuberculosis; OR = odds ratio; CI = confidence interval; NA = not available; AIDS = acquired immune-deficiency syndrome; ART = antiretroviral therapy.

This may reflect a waning in TB-specific immune responses over time in a low TB prevalence environment. One could also argue that the low risk of positive IGRA after prolonged stay in low-endemic countries might reflect more patients on ART and correspondingly higher CD4 counts, as shown in this and previous studies.[14,15] However, CD4 count and ART were adjusted for in our multivariate model, and length of stay in Norway remained significantly associated with QFT-GIT-negative results. It is also possible that some TB patients who have resided for longer periods in Norway were missed by this study if they had died due to progression from latent to active TB, complications caused by HIV infection or other causes.

It is known that the risk of progression of active TB from latent phases varies over time, being higher during the first few years, and that infection may even resolve spontaneously in some cases.[16] Wiker et al. analysed TB registry data in Norway following birth cohorts over 10-year periods before and after LTBI was brought under control.[17] Using a model based on decreasing rates of reactivated TB as an indication of waning of latency, the authors suggested a half-life of 8.8 years for LTBI in settings of low disease transmission. Our data raise the question as to whether IGRAs can be used as biomarkers of waning immune responses and thus resolution of TB infection. One obstacle to this approach is the positive IGRA results observed in patients with previous active TB as well as false-negative results, which may occur in active TB patients.[10,11,13,18] It has not been proven whether a positive IGRA after previous active TB represents inadequate TB treatment or sustained T-cell memory responses. Nevertheless, IFN-γ production often decreases after treatment, although studies of IGRA tests have not been unanimous.[19–21] There are also reports indicating that IFN-γ responses may decrease over time after TB exposure without preventive treatment, suggesting spontaneous resolution of LTBI.[22] Mori et al. found that TB prevalence measured by QFT-GIT in an older Japanese population with previous TB exposure was much lower than the predicted TB prevalence, a finding that supports our data indicating the waning of IFN-γ immune responses over time.[23]

HIV-infected patients have the highest risk of progression from latent to active TB, and there is an estimated risk reduction of 32% if preventive anti-tuberculosis treatment is given.[24,25] If a time-dependent decline in TB-specific IFN-γ responses implies clearance of LTBI without treatment, this could have policy implications for preventive anti-tuberculosis treatment guidelines in low-endemic countries. However, these results should be interpreted with caution, particularly in HIV patients, as it is still unclear whether reversion of IFN-γ responses indicates true resolution of LTBI. Longitudinal studies of longer durations are therefore needed to access the dynamics of TB-specific immune responses and the predictive value of IGRA reversion in HIV patients in low TB-endemic settings.

It is of note that none of the LTBI patients followed up in our cohort developed TB during the observation time of 24 months. In the review by Diel et al., only two studies accessed the predictive value of IGRAs for active TB development in HIV patients:[26] Aichelburg et al.'s study in Austria, in which 3/36 QFT-GIT-positive LTBI patients not on prophylactic treatment developed active TB (positive predictive value [PPV] 8.3%) within the observation time of 19 months, and Clark et al.'s study conducted in the United Kingdom in which 2/20 T-SPOT-positive untreated LTBI patients developed active TB (PPV 10%) within the observation time of 12 months.[27,28] However, further studies are needed to identify HIV patients with LTBI who are at risk for development of active TB.

In our study, the agreement between TST and IGRAs was moderate and concordance between both IGRAs was good, consistent with previous studies.[5,13,29,30] TST+/QFT-GIT− discordance was not associated with BCG status as previously reported,[31] and may be explained by the higher numbers of BCG-vaccinated subjects in our study. TST−/QFT-GIT+ discordance did not seem to be explained by T-cell anergy due to low CD4 counts. In contrast to reports from other IGRA studies in HIV patients,[13,32,33] we experienced few indeterminate results and the majority of these had high CD4 counts. We did, however, observe that IFN-γ levels were significantly higher in the TST+/QFT-GIT+ group than in the TST−/QFT-GIT+ group,[30] implying that TSTs are more often negative at QFT-GIT values close to cut-off. Although IGRAs are credited to be more sensitive than the TST in immune-compromised patients,[5,11] it is unclear whether this finding represents false-negative TST or false-positive QFT-GIT. It is of note that IFN-γ levels were also lower in discordant QFT-GIT+/T-SPOT− patients. It has been shown in studies of serial testing that QFT-GIT values close to cut-off often revert to negative or fluctuate around the cut-off values.[34] Whereas evidence for increased TB risk in TST-positive patients is reproduced by recent studies,[35] more data are necessary to define conversions and reversions and determine the predictive value of IGRA results close to cut-off.

Our study has some limitations: the QFT-GIT tests were not performed by the same laboratory; however, all seven hospitals used the commercially available QFT-GIT assay with standardised procedures. QFT-GIT results were not repeated and were therefore not checked for intra-assay variability and variance around cut-off values.[8,34]

CONCLUSION

The high prevalence of LTBI underscores the importance of TB screening in the HIV-infected population, even in low TB endemic countries. Patients originating from TB-endemic countries have a high risk of positive IGRA. However, the risk was significantly lower after several years of residence in a low TB endemic country. This may represent a decline in TB-specific immune responses due to low infectious pressure. Longitudinal studies of longer duration are necessary to further examine the dynamics of TB-specific immune responses and prognosis in HIV patients living in low TB burden areas.

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RÉSUMÉ

OBJECTIFS : Déterminer la prévalence de la tuberculose (TB) et le caractère prédictif positif des tests de libération de l'interféron gamma (IGRA) et du test cutané à la tuberculine (TST) chez des patients positifs pour le virus de l'immunodéficience humaine (VIH) dans un pays de faible endémicité, la Norvège.

SCHÉMA : Etude multicentrique transversale sur 298 patients VIH positifs testés par QuantiFERON TB-Gold In-Tube® (QFT-GIT), T-SPOT®.*TB* (T-SPOT) et au TST.

RÉSULTATS : Au total 77/298 tests QFT-GIT (26%), 29/117 T-SPOT (25%) et 52/217 TST (⩾5 mm ; 24%) se sont avérés positifs. Le taux médian de CD4 était de 427 cellules/μl. Trois tests QFT-GIT, mais aucun T-SPOT ont eu un résultat indéterminé. Sur les 52 patients dont le TST était positive, 34 (65%) avaient un QFT-GIT positif (médian IFN-γ = 4,38 UI/ml) tandis que 16% des patients TST-négative avaient un QFT-GIT positif (médian IFN-γ 0,81 UI/ml, $P > 0{,}001$). Un QFT-GIT positif était associé à une origine dans un pays d'endémie tuberculeuse, une TB active antérieure et une exposition à la TB ($P \leqslant 0{,}01$). Les patients venus de zones d'endémie mais installés en Norvège depuis au moins 10 ans avaient une moindre probabilité d'avoir un QFT-GIT positif (12% ; OR 0,17 ; IC95% 0,06–0,53 ; $P = 0{,}002$) que les patients arrivés depuis moins de 3 ans (49%).

CONCLUSION : La prévalence d'IGRA positifs chez des patients VIH positifs s'est avérée élevée dans ce contexte de faible endémicité. La diminution de positivité du QFT-GIT chez des patients ayant longtemps vécu en Norvège pourrait refléter un déclin des réponses immunitaires spécifiques de la TB.

RESUMEN

OBJETIVO: Determinar la prevalencia y los factores pronósticos de un resultado positivo de las pruebas de liberación de interferón gama (IGRA) y de la reacción a la tuberculina en los pacientes infectados por el virus de la inmunodeficiencia humana (VIH) en Noruega, un país con baja endemia de tuberculosis (TB).

MÉTODOS: Fue este un estudio transversal multicéntrico de 298 pacientes infectados por el VIH en quienes se practicó la prueba QuantiFERON TB-Gold en Tubo® (QFT-GIT), la prueba TB-SPOT®.*TB* (T-SPOT) y la reacción cutánea a la tuberculina (TST).

RESULTADOS: Se obtuvieron resultados positivos en 77 de los 298 pruebas QFT-GIT (26%), en 29 de los 117 T-SPOT (25%) y en 52 de los 217 TST (24%; ⩾5 mm). La mediana del recuento de células CD4 fue 427 células/μl. Se obtuvieron tres resultados no concluyentes con la prueba QFT-GIT, pero ninguno con el ensayo T-SPOT. De los 52 pacientes con TST positiva, 34 presentaron una QFT-GIT positiva (65%; mediana 4,38 UI/ml de INF-γ) y solo 16% de los pacientes con una TST negativa obtuvieron un resultado positivo con el ensayo QFT-GIT (mediana de 0,81 UI/ml de INF-γ; $P < 0{,}001$). Los factores que se asociaron con un resultado positivo de la QFT-GIT fueron la procedencia de un país con TB endémica, el antecedente de TB activa y la exposición a un caso de TB ($P \leqslant 0{,}01$). Los pacientes procedentes de países con TB endémica que habían residido en Noruega durante ⩾10 años presentaron menor probabilidad de una prueba QFT-GIT positiva (12%; OR 0,17; IC 95% 0,06 a 053; $P = 0{,}002$), en comparación con los pacientes que habían residido menos de 3 años en el país (49%).

CONCLUSIÓN: La prevalencia de resultados positivos con las IGRA fue alta en este entorno con baja endemia de TB. Los resultados positivos más bajos en las pruebas QFT-GIT de los pacientes procedentes de países endémicos con una estadía prolongada en Noruega refleja una disminución progresiva de la respuesta inmunitaria específica a la TB.