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Outbreak of Transient Conversions of the QuantiFERON-TB Gold In-Tube Test in Laboratory Health Care Worker Screenings

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Gamma interferon release assays were recently introduced in health care worker (HCWs) screenings for tuberculosis surveillance. In longitudinal surveys, conversions and reversions are seen, and yet whether these changes are unspecific or are an expression of new infections and microbial clearance remains unclear. In order to further elucidate these changes, we analyzed an outbreak of 15 transient conversions in 53 HCWs who operate in the same laboratory and handle specimens potentially containing *Mycobacterium tuberculosis* who underwent screening by the QuantiFERON-TB Gold In-Tube (QFT-GIT) test between 11 May and 30 June 2010: 15/46 (33%) negative HCWs showed a conversion and then reverted after 7 to 107 days. To validate these results, an evaluation of methodological procedures and test reliability, as well as an analysis of results obtained during the same period and processed by the same laboratory, was carried out. For the latter purpose, QFT-GIT results determined for 78 ward HCWs who underwent screening during the same period and were employed in departments with at least 3 infectious tuberculosis patients per year or had cared for an infectious patient without airborne precautions were analyzed with the following results: 6/63 (9%) HCWs with negative results in 3 different departments showed transient conversion ($P = 0.002$; odds ratio, 4.60; 95% confidence interval, 1.62 to 13.04). A retrospective survey of in-house biosafety practices led to determination of a single exposure factor within the laboratory. These data emphasize the validity of the hypothesis that a transient conversion demonstrates the presence of a real tubercular infection and could be an important indicator for occupational biosafety concerns. They also confirm that subjects with recent conversion should be retested before chest radiography and chemotherapy is offered.

Health care workers (HCWs) are at a higher-than-average risk of tuberculosis (TB). In countries with low (<50 cases/100,000 population), intermediate (50 to 100 cases/100,000 population), and high (>100 cases/100,000 population) TB incidence, estimated incident rate ratios (RR) for active TB in HCWs are 2.4, 2.4, and 3.7, respectively (3). That report also states that sound TB infection control measures might decrease TB annual incidence among HCWs by as much as 49%, 27%, and 81% in countries with low, intermediate, and high incidence, respectively. Among laboratory-acquired infections, TB is the most common (10). Because of this high risk, international guidelines recommend serial screening of HCWs in order to detect latent tubercular infection (LTBI) (6).

For diagnosing LTBI, the only test in use until recently was the tuberculin skin test (TST), despite its many limitations (15). Two gamma interferon (IFN-γ) release assays (IGRAs) have recently emerged as promising alternatives to TST. One measures the level of IFN-γ produced by activated effector lymphocytes stimulated with *Mycobacterium tuberculosis*-specific antigens (QuantiFERON-TB Gold In-Tube [QFT-GIT]; Cellestis Limited, Carnegie, Victoria, Australia), while the other measures the number of T lymphocytes producing IFN-γ detected after *M. tuberculosis*-specific antigen stimulation (T-SPOT.TB; Oxford Immunotec Limited, Abingdon, United Kingdom). IGRAs overcome several of the TST limitations: they are uninfluenced by previous *M. bovis* BCG vaccination and by nontubercular mycobacteria (NMT) other than *M. kansasii*, *M. szulgai*, and *M. marinum*; they enable objective reading of the results and evaluation of the immune system function due to the presence of positive and negative controls; no boosting phenomenon or sensitization occurs, since they are *ex vivo* tests; and as a consequence of the absence of side effects and the need for only a single access, they ensure better compliance.

Additionally, IGRAs prove more accurate than TST in LTBI diagnosis (8) and show better association with the gradient of exposure in contact investigations (1, 22). In low-incidence countries, IGRAs detect lower prevalences of LTBI in HCWs, probably as a consequence of the greater specificity of the tests in BCG-vaccinated people, and better association with occupational risk (33).

Serial testing of HCWs, however, has shown that IGRAs are extremely dynamic, with high rates of conversions and reversions (33). A key unresolved issue remains the interpretation of these changes.

A true conversion is defined as an increase in IFN-γ production after challenge with tubercular antigens that is above the variations of technical and biological noise, reflecting the presence of a new tubercular infection. A spontaneous reversion is a very important observation, potentially representing microbial clearance; however, the response decrease should clearly exceed the method's total imprecision.

The analytical precision of QFT-GIT has proven to be good (7, 25, 28), in contrast, intraindividual variability has not yet been adequately assessed.

A study from a high-burden country (30) showed 16% variability for 14 HCWs with both positive and negative QFT-GIT results after the HCWs were tested 4 times in a 12-day period.

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A report from Germany (25) conducted on 35 prevalently positive nurses tested from 3 to 5 times weekly indicates findings that represented wide variability (67%).

Finally, a study from South Africa (29) performed with 26 subjects tested 4 times weekly showed a variability of 80%, leading to the hypothesis that spontaneous conversions and reversions can potentially occur during serial testing over a short time period, even in the apparent absence of any exposure. That study, however, was conducted in a high-burden country, where unrecognized exposures might occur. Such findings raise the issue of whether conversions and reversions identified in HCW serial screenings are the result of random variations around the cutoff level or whether they reflect different stages in the spectrum of tuberculosis infection (3, 21).

This is a very relevant issue, because if the inconsistencies in IGRA results are unspecific, their detection is misleading, since they lead to unnecessary investigation of potential infection transmission, in-depth medical evaluation of involved subjects, and repeated testing, as well as wasted administrative and other measures.

In contrast, if changes in IGRA results are actually related to new infections and bacillus clearance, their detection in HCWs is a reliable tool for identification of deficiencies in infection control programs and for strengthening protective measures. Moreover, the kinetics of IGRA responses could help clarify the dynamics of TB infection (9, 11, 18–21, 29, 31).

Data on this issue are difficult to interpret because of the lack of a gold standard for the diagnosis of LTBI. Furthermore, studies are limited and come from heterogeneous settings (29, 33); thus, a need exists for additional knowledge.

This observational study is based on the results of LTBI screening conducted with HCWs at risk of exposure to *M. tuberculosis* at an academic tertiary care center in northern Italy in accordance with international guidelines (12, 13, 14) and with Italian law (23, 24).

The objective was to analyze an outbreak of transient conversions of QFT-GIT observed in a group of laboratory HCWs over a 7-week period to add evidence to support one or the other of the above-mentioned hypotheses.

MATERIALS AND METHODS

The present study was conducted at the University Hospital of Padua, Padua, Italy. Data were collected from the medical records of HCWs included in the TB surveillance program under the supervision of the Occupational Medicine Section of the institution, according to the criteria established by the Centers for Disease Control and Prevention (12) and Italian law (23, 24).

Since 2007, the test in use has been the QFT-GIT, performed at the same institution by an accredited laboratory which is the regional referral center for diagnostics on mycobacteria.

The QFT-GIT test is processed in accordance with the manufacturer's specifications (5). Whole blood is drawn into 3 tubes: a nil control tube containing no antigens (negative control [Nil]), a mitogen control tube containing nonspecific T-cell-stimulating antigen (positive control), and an *M. tuberculosis* antigen tube containing a pool of *M. tuberculosis*-specific antigens. After thorough mixing, the tubes are incubated at 37°C for 16 to 24 h. The tubes are then centrifuged for 15 min at 3,000 rpm and stored at 4°C until the assay is performed, 2 to 4 days after collection. The amount of IFN-γ released is measured by an enzyme-linked immunosorbent assay (ELISA). The test result is considered positive if the IFN-γ response of *M. tuberculosis* antigens minus Nil is ≥0.35 IU/ml. The same analytical session provides the determination of IFN-γ levels in samples drawn from HCWs and from inpatients and outpatients.

HCWs from 3 distinct work groups underwent the QFT-GIT test in the period under study. All were tested with the same instrumentation by the same accredited laboratory.

The first group involved HCWs employed in a laboratory which processes clinical specimens that may contain *M. tuberculosis* who underwent the routine annual LTBI screening between 11 May and 30 June 2010. An unexpectedly high number of them exhibited new positive QFT-GIT results.

To check whether a technical anomaly produced those unexpected results, the same blood specimens were processed using a different lot of reagents and a second measuring device.

In the same period, a second group of HCWs, involved in care activities, underwent the QFT-GIT serial screening. They belonged to various departments and included those working in medium-risk departments according to CDC criteria, those exposed to infectious patients without airborne precautions, and those who had carried out in-depth health checks. Results of QFT-GIT screening of laboratory HCWs and ward HCWs were compared to check whether a systematic failure in the test that had gone unrecognized (either by the performer laboratory or by the producer quality control system) had produced the conversions.

By coincidence, a third group of HCWs was tested with the same QFT-GIT batch number during the same time period: they worked at a different hospital and had had unprotected exposure to an infectious TB patient. Their results were compared to those of laboratory HCWs as well, in order to further validate the data.

The laboratory and ward HCWs with QFT-GIT conversion underwent repeated testing, according to the 2010 CDC guidelines (14) that suggest result confirmation in persons with discordant QFT-GIT.

All converted HCWs underwent a full medical evaluation, including history and physical examination, to obtain information as to contact with cases of active TB outside the work environment and respiratory signs or symptoms of active tuberculosis.

In the case of asymptomatic, physical examination-negative HCWs, chest radiography was delayed until repeated testing confirmed conversion, according to the 2010 CDC guidelines (14) that recommend deferment of diagnosis for persons with discordant test results, unless an increased risk exists for progression if infected and/or for a poor outcome if disease develops.

Laboratory HCWs were retested between 6 September and 5 October 2010 and finally between 2 January and 15 March 2011, according to CDC guidelines (12) for cases of outbreak detection.

Ward HCWs were tested afterwards according to a standard surveillance program.

The third group of HCWs, those working at a different hospital, was retested in September 2010, according to the two-step follow-up indicated by the CDC (12) for cases of unprotected work exposure.

With the aim of recognizing a source of exposure to *M. tuberculosis*, a series of investigations were conducted in the laboratory where the outbreak of conversions occurred.

QFT-GIT laboratory HCWs with previously stable positive results underwent evaluation by history, physical examination, and chest radiography by an experienced radiologist to investigate whether a person with unrecognized active TB infection was spreading mycobacteria in the working environment.

To the same purpose, a group of HCWs occasionally working at the laboratory (not included in the laboratory group or in the hospital ward group) underwent testing to detect cases with positive QFT-GIT results and eventually subject them to further examination.

Experienced technicians conducted environmental control tests, in particular, in air conditioning systems, to uncover possible sources of contamination with *M. marinum* or *M. szulgai* or *M. kansasii*, each of which cross-reacts with specific antigens in the QFT-GIT screen (16).

Practices and procedures in the laboratory, particularly the appropriateness and the degree of adherence to the infection control plan, were

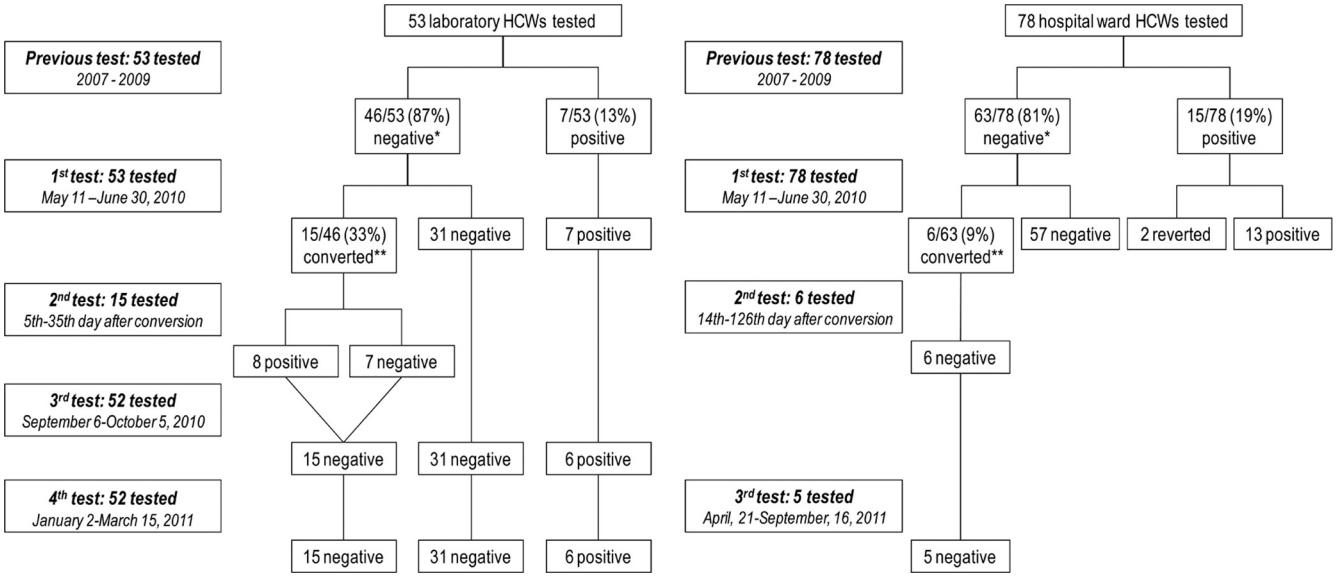

FIG 1 Flow diagram for 53 laboratory health care workers (HCWs) and 78 ward HCWs screened for latent tubercular infection with the QuantiFERON-TB Gold In-Tube test. *, $P = 0.36$ (two-sided z-test); ** $P = 0.002$ (two-sided z-test); OR, 4.6 (CI, 1.62 to 13.04).

reassessed to identify lapses that could have led to contamination and transmission.

Statistical methods. Data analysis was performed using Statgraphics Centurion XV (StatPoint, Inc., version 15.1.02). Means of IFN-γ levels were compared, including calculating the 95% confidence intervals (CIs) based on Fisher's Least Significant Difference (LSD) procedure. The mean ages of the two groups of laboratory and ward HCWs were compared using the two-sided *t* test.

The proportions of negative subjects and of transiently converted subjects, in the two HCW groups, were compared using the two-sided z-test, and 95% CIs were calculated (Mid-P Exact).

Relations of the results were determined and codified as odds ratios (OR; Taylor series) between transiently converted subjects in the two HCW groups.

No approval of an ethics commission was required, since data collection was performed during mandatory screenings according to Italian work protection laws (23, 24). No additional tests were performed for study purposes, and it is not necessary to request ethical approval for the anonymous analysis of routine data in Italy.

RESULTS

Overall, this report compares the results determined with 174 samples drawn from laboratory HCWs, 89 samples from ward HCWs, and 64 samples from HCWs working at a different hospital between 11 May and 30 June 2010.

A total of 131 HCWs working at the University Hospital of Padua underwent the QFT-GIT screen in the period under study: 53 from a laboratory and 78 from different care departments at the same institution.

Figure 1 illustrates the number of subjects included at each step of the study in the 2 groups.

For 15 out of 46 laboratory HCWs with previously (2007 to 2009) negative QFT-GIT results, conversion was detected (33%; 95% CI, 20.3 to 47.1). For 12 cases, the conversion was confirmed by the repetition of the QFT-GIT screen on the same sample. Three specimens were not retested because of organizational constraints.

A few days later, the 15 converted laboratory HCWs underwent a second test: 7 were found to have reverted at between 7 and 35 days after conversion (2 underwent an extra test after 4 weeks, which was again negative), whereas the test results for 8 remained positive. Results of repeat tests administered to the 15 laboratory HCWs in September to October 2010 and January to March 2011 were all negative.

In the group of 78 ward HCWs who underwent the QFT-GIT screen between 11 May and 30 June 2010, 6 of the 63 that had previously tested negative showed conversion (9%; CI, 3.9 to 18.8). The difference between the number of HCWs who converted in the laboratory group (15/46) and the number of those who converted in the ward group (6/63) was statistically significant ($P = 0.002$; OR, 4.6; CI, 1.62 to 13.04). The general characteristics of the 53 laboratory and of the 78 hospital ward HCWs did not exhibit relevant differences (Table 1).

Ward HCWs were employed in different departments; thus, no

TABLE 1 Demographic characteristics of 131 HCWs who underwent a QuantiFERON-TB Gold In-Tube test between 11 May and 30 June 2010

Characteristic	No. (%) of laboratory HCWs	No. (%) of ward HCWs
Gender		
Male	13 (25)	20 (26)
Female	40 (75)	58 (74)
Age		
Mean	46[a]	42[a]
SD	8.5	9.8
Job title		
Nurse, HC assistant	12 (23)	72 (93)
Technician	34 (64)	1 (1)
Doctor	7 (13)	5 (6)
Total	53	78

[a] $P = 0.013$ (two-sided *t* test).

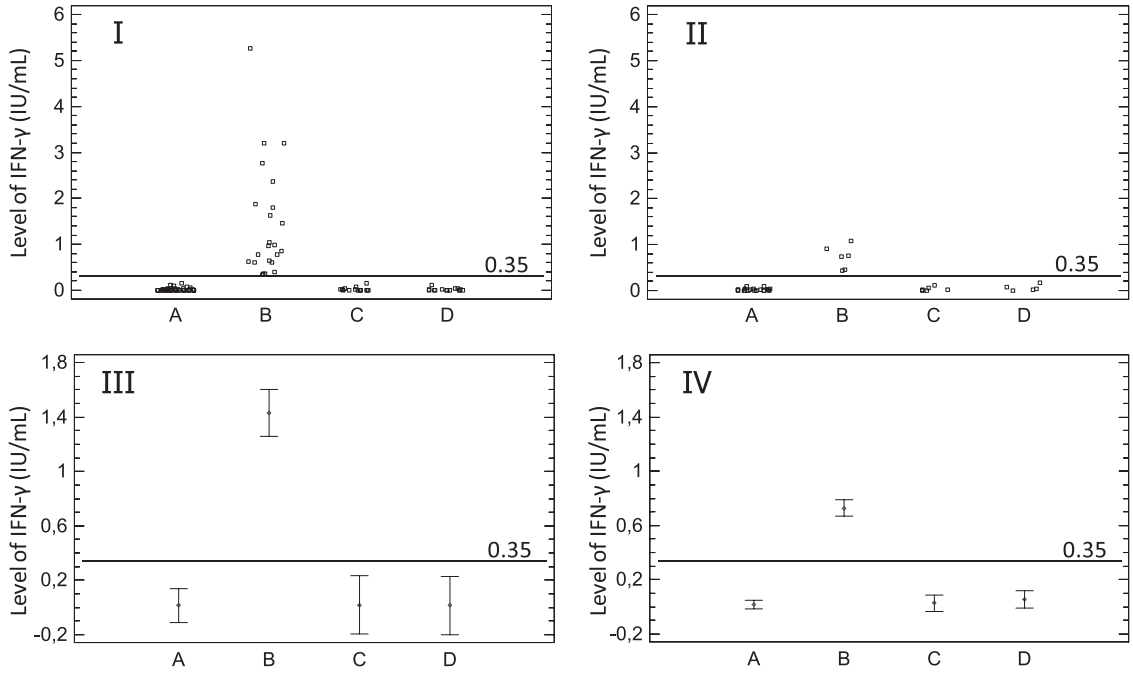

FIG 2 Interferon-γ (IFN-γ) level comparisons for 15 laboratory health care workers (HCWs) (I and III) and 6 ward HCWs (II and IV) who showed transient conversions in QuantiFERON-TB Gold In-Tube testing between 11 May and 30 June 2010. In panels III and IV, data represent means with 95% LSD intervals. Series A consisted of tests performed in previous routine screening (2007 to 2009). Series B consisted of tests performed between 11 May and 30 June 2010 (positive values). Series C consisted of tests performed between 6 September and 5 October 2010 (laboratory HCWs) and between 6 July and 2 November 2010 (ward HCWs). Series D consisted of tests performed between 2 January and 15 March 2011 (laboratory HCWs) and between 21 April and 16 September 2011 (ward HCWs).

outbreak of transient conversions was evidenced. Of note, all 6 of the ward HCWs who transiently converted belonged to the group of those who were tested because they had cared for at least one infectious TB patient without airborne precautions. One patient care assistant attended a smear-positive patient in the Pediatric Department from 31 March to 9 April 2010. Three nurses assisted a smear-positive patient in an internistic ward from 13 December 2009 to 29 January 2010. A physician and a nurse performed an outpatient bronchoscopy on 29 March 2010 that afterwards gave an *M. tuberculosis* culture-positive result. These 6 HCWs had repeated testing from 14 to 126 days after the first test: all had negative test results, and these results were confirmed between April and September 2011.

Only 1 of the 64 HCWs working at another hospital who by coincidence were tested after an unprotected exposure to an infectious patient with the same QFT-GIT batch number, showed a conversion and returned negative test results in September 2010.

Figure 2 shows IFN-γ values of negative and positive tests of 15 laboratory and 6 hospital ward HCWs with transient conversions, in different periods; summary statistics are illustrated in Table 2.

Table 3 shows IFN-γ values of 5 laboratory HCWs with 1 positive QFT-GIT (QFT1) result and a subsequent negative test result (QFT2); previous TST (5 IU) results, collected from occupational medicine clinical records, are also reported.

Table 4 quotes IFN-γ values of 8 laboratory HCWs with 2 subsequent positive QFT-GIT (QFT1 and QFT2) test results; previous TST (5 IU) results are also reported.

Although a difference exists between IFN-γ values in the two groups, it is not statistically significant (mean of IFN-γ values in Table 3, 0.93 IU/ml [CI, 0.37 to 1.50]; mean of IFN-γ values in Table 4, 1.61 IU/ml [CI, 1.26 to 1.97]).

One laboratory HCW had borderline values in two replicated tests (0.27 and 0.36 IU/ml) on 27 May 2010 and then, on 1 July 2010, returned negative test results (0.00 IU/ml). Another laboratory HCW, at QFT-GIT testing on 14 June 2010, exhibited a value that was still negative but higher than those determined in previous years (0.29 IU/ml); he then received repeat testing on June 24 2010 that showed conversion (1.8 IU/ml) and subsequently, on 6 September 2010, turned negative (0.01 IU/ml).

TABLE 2 Summary statistics for IFN-γ levels in 15 laboratory and 6 ward HCWs who showed transient conversions of the QuantiFERON-TB Gold In-Tube test between 11 May and 30 June 2010[a]

Test series	No. of samples	IFN-γ level (IU/ml)						
		Mean	95% CI lower limit	95% CI upper limit	SD	Median	Minimum	Maximum
Laboratory HCWs								
A	45	0.02	−0.11	0.14	0.03	0.00	0.00	0.15
B	23	1.43	1.26	1.60	1.22	0.96	0.35	5.27
C	15	0.02	−0.19	0.23	0.04	0.00	0.00	0.15
D	15	0.01	−0.20	0.23	0.03	0.00	0.00	0.12
Hospital ward HCWs								
A	20	0.02	−0.01	0.05	0.03	0.01	0.00	0.10
B	6	0.73	0.67	0.79	0.25	0.75	0.43	1.07
C	6	0.03	−0.03	0.09	0.04	0.01	0.00	0.11
D	5	0.06	−0.01	0.12	0.07	0.03	0.00	0.17

[a] For an explanation of test series A, B, C, and D, see the Fig. 2 legend.

TABLE 3 IFN-γ levels in 5 laboratory health care workers whose QuantiFERON-TB Gold In-Tube test results reverted to negative upon additional testing[a]

Subject	Date (mo/day/yr)	No. of IU/ml	Serial no. of QFT GIT	No. of previous TST-positive results (mo/day/yr of test) or mm of induration
1	05/27/2010	0.36	QFT 1	0 (05/11/2004)
	05/27/2010	0.45	QFT 1	
	06/03/2010	0.07	QFT 2	
	06/03/2010	0.19	QFT 2	
2	06/11/2010	0.63	QFT 1	0 (01/11/2002)
	06/11/2010	0.68	QFT 1	
	07/08/2010	0.02	QFT 2	
3	06/11/2010	0.77	QFT 1	0 (04/27/2004)
	06/11/2010	0.86	QFT 1	
	06/28/2010	0.01	QFT 2	
4	06/11/2010	0.99	QFT 1	7 mm[b] (01/26/2001)
	06/11/2010	1.10	QFT 1	
	06/28/2010	0.12	QFT 2	
5	05/31/2010	1.87	QFT 1	None
	05/31/2010	1.63	QFT 1	
	06/07/2010	0.31	QFT 2	

[a] Previous tuberculin skin test (TST) (5 IU) results are also reported (expressed as millimeters of induration). The same date is shown for tests replicated on the same specimen; cutoff, 0.35 IU/ml.
[b] BCG vaccinated on 03 February 1989 and 26 November 1997.

TABLE 4 IFN-γ levels in 8 laboratory health care workers whose positive QuantiFERON-TB Gold In-Tube test results were confirmed upon additional testing[a]

Subject	Date (mo/day/yr)	No. of IU/ml	Serial no. of QFT GIT	No. of previous TST-positive results (mo/day/yr of test) or mm of induration
6	05/31/2010	0.35	QFT 1	0 (05/10/2004)
	05/31/2010	0.42	QFT 1	
	06/28/2010	0.40	QFT 2	
7	05/27/2010	0.61	QFT 1	0 (05/11/2004)
	05/27/2010	0.49	QFT 1	
	05/27/2010	0.40	QFT 1	
	06/03/2010	0.61	QFT 2	
	06/03/2010	0.36	QFT 2	
8	06/03/2010	0.96	QFT 1	8 mm (01/16/2001)
	06/09/2010	1.04	QFT 2	
9	05/31/2010	0.64	QFT 1	0 (03/23/2001)
	05/31/2010	0.64	QFT 1	
	06/07/2010	2.36	QFT 2	
	06/07/2010	2.41	QFT 2	
10	05/27/2010	1.46	QFT 1	6 mm[b] (01/26/2001)
	05/27/2010	0.83	QFT 1	
	06/03/2010	0.86	QFT 2	
11	06/10/2010	2.76	QFT 1	6 mm[c] (01/23/2001)
	06/22/2010	0.78	QFT 2	
12	06/11/2010	3.19	QFT 1	None
	06/11/2010	3.92	QFT 1	
	06/23/2010	3.20	QFT 2	
13	05/26/2010	5.27	QFT 1	9 mm (02/06/2001)
	05/26/2010	4.65	QFT 1	
	05/31/2010	1.62	QFT 2	
	05/31/2010	1.76	QFT 2	

[a] Previous tuberculin skin test (TST) (5 IU) results are also reported (expressed as millimeters of induration). The same date is shown for tests replicated on the same specimen; cutoff, 0.35 IU/ml.
[b] BCG vaccinated on 19 January 1995.
[c] BCG vaccinated on 29 June 1995.

Ten of 15 laboratory HCWs exhibited values of positive QFT-GIT results higher than 0.70 IU/ml.

Two of 15 had values belonging to the "borderline zone" between 0.2 and 0.7 IU/ml (20) 7 days before or after a test with higher values; in one case, it was the negative value that fell into the "borderline zone"; in the other case, it was the positive value that fell into that zone.

Note that, in the period in which transient conversions were detected, a previously QFT-GIT-positive laboratory HCW exhibited a marked increment of increase, similar to a booster effect, in the IFN-γ level (the values determined from 2007 to 2009 were 0.55, 0.19, 0.47, and 0.38 IU/ml; the value on 11 June 2010 was 9.65 IU/ml; the value on 28 September 2010 was 0.79 IU/ml; and the value on 26 January 2011 was 1.18 IU/ml).

At medical evaluation, none of the 15 converted QFT-GIT laboratory HCWs reported contact with cases of active TB among family members, friends, or acquaintances. One reported a recent cough and consequently underwent chest radiography. The 8 colleagues who had 2 subsequent positive tests, although asymptomatic, underwent chest radiography as well. All 9 radiograms were negative.

None of the 7 laboratory HCWs with previously stable positive QFT-GIT results had, at the time of their ITBL detection, accepted chemotherapy; thus, although asymptomatic, they underwent chest radiography again, which in no case revealed active TB.

Of 10 HCWs occasionally working at the laboratory, none had positive QFT-GIT results.

No environmental source of *M. szulgai* or *M. marinum* or *M. kansasii* was detected in the laboratory, and no specimens containing these mycobacteria were identified in a retrospective evaluation of samples handled in the previous 6 months.

In contrast, the retrospective investigation of operating procedures revealed that, in April 2010, one sample initially not suspected of containing *M. tuberculosis* was processed, out of a class II biological safety cabinet, in all working areas where the 15 transiently converted laboratory HCWs worked. The sample was found afterward to be positive for *M. tuberculosis* by culture and microscopy. Hence, unnoticed environmental contamination, with spread of mycobacteria, most likely occurred.

DISCUSSION

This report illustrates an outbreak of 15 transient QFT-GIT conversions in a group of 53 laboratory HCWs, 46 of whom had always tested negative in the previous years. Interestingly, no such instance was observed in the other 2 groups, which consisted of 78 ward HCWs working at the same institution and 64 HCWs working at another hospital.

This was the first time that such an outbreak was detected at the hospital under study, in a 4-year period of TB surveillance of HCWs by the use of QFT-GIT testing.

It was first thought that these data could have been spurious, resulting from analytical error, a systematic decrease of test specificity, random variation of laboratory results, or unspecific intra-individual variations.

None of these was found to be true.

The literature indicates very good accuracy of the QFT-GIT screen (7, 25, 28, 29), and, in fact, the personnel in the laboratory that performed the QFT-GIT screen are skilled in these procedures and performed all controls for the verification of acceptance criteria and accuracy of results without detecting anomalies.

The consistency of 12 replicated tests performed on the same specimens with different batches of reagents and measuring instruments ruled out analytical error as well.

Quality controls requested from the manufacturer revealed no decrease in test specificity.

Seven HCWs had 2 tests in the same 7-week time interval, with a positive and a negative result, negating the possibility of a systematic decrease in test specificity as well.

Comparison with a control cohort revealed a lower rate of transient conversion (6/63; 9%) in ward HCWs from the same hospital who had previously had negative test results. It might also be argued that 9% is a rather high rate of conversion, but these 6 ward HCWs worked in 3 different departments. Moreover, this percentage refers to the 78 ward HCWs that, by chance, were given the test between 11 May and 30 June 2010. This group is a part of the total number of ward HCWs employed in departments at medium risk or who had unprotected exposure and underwent the QFT-GIT screen also in previous and subsequent weeks.

In a second group of 64 HCWs from a different hospital, who underwent the test in the same 7-week period and were analyzed using reagents with same batch number, only one (2%) showed transient conversion.

These findings do not support the hypothesis of a transient systematic decrease in test specificity or that of random variations of the laboratory results.

The transient conversions recorded in this study might have been unspecific results owing to within-subject variations in IFN-γ production unrelated to mycobacterial infection. Some surveys, indeed, testify to considerable intraindividual variability (25, 29). A 4-week survey of 35 German HCWs working in different departments, selected on the basis of previous IGRA or TST positivity, with no ongoing TB exposure, revealed 6 reversions and 4 transient conversions by QFT-GIT testing (25).

The case of the present study is quite different, however. The conversions involved a group of workers who shared work practices and working environment. None of the 15 transiently converted laboratory HCWs had prior QFT-GIT positivity. In most cases, even previous TST results provide no evidence of preexisting TB infection. Hence, the results of the study from Germany and those of this study may represent different underlying phenotypes of latent tubercular infection (21).

Additionally, simultaneous unspecific within-subject variations in 15 of 46 HCWs that always proved negative on tests repeated in 3 previous years, as well as in subsequent testing, are hardly conceivable. Statistical analysis supports this belief, demonstrating that the negative values detected at previous and subsequent testing and the positive values definitely belong to different populations (Fig. 2).

Thus, these findings provide support for the hypothesis that transient conversions of IGRA may be due to actual changes in the immune response to *M. tuberculosis* subsequent to new infection followed by pathogen clearance.

This occurrence has already been described (9, 11, 18–21, 29, 31). The existence of acute TB infections that "self-cure" has been known since the studies were performed in which the TB infection was sought by TST (17), and recent studies highlight how the spectrum of tubercular infection is much more complex than the simple dichotomy of latent infection-clinical disease (2). In particular, infection seen in association with T cell priming may be eliminated at an early stage (2, 32). In the present study, this occurrence is defined as "transient tubercular infection" (17).

Most studies that have addressed serial IGRA testing in HCWs have relied on limited longitudinal data, i.e., a series of 2 or 3 tests performed at intervals ranging from 6 months to 4 years (33), providing only limited information on the kinetics of the underlying phenomena, and thus not adequately clarifying their nature.

This report extends previous observations in that the 15 HCWs involved in transient conversions had at least 7 subsequent tests, 3 tests prior to conversion, and 3 tests afterward, hence furnishing a larger set of data to explore the dynamics of the underlying transitions.

To reduce the influence of within-subject variability and improve the interpretation of QFT-GIT results in serial testing, a "borderline zone" between 0.2 and 0.7 IU/ml has been proposed (20, 27, 29, 30): variations in test results maintained within this range should be cautiously considered to distinguish expression of true conversion from reversion of the test. Only 5 of 15 laboratory HCWs with transient conversion showed positive tests constrained within 0.7 IU/ml, whereas 10 exhibited higher values. Consequently, use of the "borderline zone" criterion would have reduced the number of HCWs classified as transiently converted, but the outbreak would have persisted: among the laboratory HCWs, 10 of 46, i.e., 22% (CI, 11.6 to 35.3), would have been classified as having converted; among the ward HCWs, 4 of 63, i.e., 6% (CI, 2.0 to 14.6), would have been classified as having converted ($P = 0.018$; OR, 4.1 [CI, 1.2 to 14.0]).

No infectious TB case was recognized among HCWs who worked at the laboratory, regularly or occasionally. The possibility exists that a laboratory HCW with ITBL spread mycobacteria in the work environment (4), but as yet this remains theoretical.

None of the 15 laboratory HCWs reported the presence of TB cases among family members, friends, or acquaintances. The laboratory under study is in Padua, Italy, where the prevalence of TB infection within the province is low, with 64 cases reported in the year 2009 among the 927,730 residents (26; ISTAT [Istituto Nazionale di Statistica], Rome, Italy [http://demo.istat.it/pop2010/index.html; 23 November 2011, accession date]). Therefore, simultaneous contact of the 15 laboratory HCWs with infectious TB cases outside the work environment is unlikely.

The conversions involved a relatively small and highly homogeneous group of workers; a lapse was identified in infection control practice in the laboratory that may have caused unnoticed environmental contamination in all the operating areas where the 15 laboratory HCWs with transient conversions worked. Thus, work-associated transmission of tubercular infection is the most probable cause.

This study suffers from some weaknesses.

This was an observational rather than a planned study and was based on analysis of data collected in the course of the institutional activities of the Occupational Health Service for surveillance of HCWs at risk of TB exposure. Because of that, the sequence of tests and time intervals were influenced by organizational constraints and were not always strictly planned.

This study compared groups of HCWs at risk of TB infection, although in differing ways; it lacked a control group with abso-

lutely no exposure. However, this particular bias would produce a reduction of risk ratings for the laboratory HCWs rather than an increase as seen in this study.

But such limitations cannot be the explanation of the present findings.

Conclusions. The present report adds evidence that transient conversions of HCWs in QFT-GIT testing may represent the expression of actual occurrences of transient TB infections which self-cure. Thus, increasing or decreasing IFN-γ production may reflect the different spectra of responses to the TB infection and not simply biological noise.

It might be argued that detection of transient conversions adds to the clinical work load with no real benefit, since they are changes with no clinical relevance. In our opinion, this is not the case in the field of occupational medicine. Occupational hazard prevention and control are accomplished with programs that embrace engineering and workplace practice controls, use of personal protective equipment, training, and education and are completed by exposure monitoring. In health care settings where workers are at risk of TB exposure, environmental measurements of viable mycobacteria are not feasible. Instead, observation of transient conversions, if confirmed as expressions of true transient tubercular infections, can be a reliable tool in assessing exposure and identifying lapses in biosafety practices or other unnoticed sources of infection transmission.

These findings also suggest that transient TB infections may be common in HCWs in low-prevalence countries as a result of exposure in the workplace, confirming that subjects with recent conversions should be retested before being offered chest radiography and chemotherapy.

Further studies and research are needed, exploring the dynamics of IGRAs in serial testing in large prospective cohorts in different settings.

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