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Original Article

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Comparação do teste de libertação do interferão-γ e da prova de tuberculina no rastreio de profissionais de saúde

Comparison of interferon-γ release assay and tuberculin test for screening in healthcare workers

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Resumo

Os profissionais de saúde (PS) têm um risco aumentado de tuberculose. O rastreio da tuberculose latente e da tuberculose activa é portanto essencial nos programas de controlo de infecção. Entre Maio de 2007 e Abril de 2009, foram utilizados simultaneamente a prova de tuberculina (PT) e o teste de libertação do interferão-γ (IGRA) em 1686 PS. Quando PT ≥10mm ou IGRA positivo, e em PS com contacto com tuberculose ou sintomáticos, foi realizada uma radiografia torácica para excluir tuberculose activa. O IGRA foi positivo em 33,1% e a PT foi >10mm em

Abstract

Healthcare workers (HCWs) have an increased risk of tuberculosis (TB). Screening for latent tuberculosis infection and active TB is therefore essential in infection control programs. Tuberculin skin test (TST) and Interferon-γ Release Assay (IGRA) were used simultaneously in 1686 HCWs between May 2007 and April 2009. A chest X-ray was performed in order to exclude active TB when TST was ≥10mm or IGRA was positive and in HCWs with TB contact or symptoms. IGRA was positive in 33.1% and TST was >10mm in 78.3% of the HCWs. The proportion

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78.3% dos PS. A proporção de resultados IGRA positivos aumentou com o diâmetro da PT. Nos PS com PT >15mm, 49.2% são IGRA positivos. A PT foi positiva em mais do dobro dos casos do IGRA. Assim, foram observados mais frequentemente resultados PT+/IGRA- do que resultados concordantes negativos ou positivos. Em nenhum PS com resultado PT+/IGRA- foi diagnosticada tuberculose activa durante o período do estudo. A vacinação repetida pelo BCG aumentou o número de casos discordantes PT+/IGRA-. Quanto menor o intervalo após a vacinação pelo BCG, maior a discordância PT+/IGRA-.
Na população de PS rastreada, foram diagnosticados 9 casos de tuberculose activa, sendo todos PT e IGRA positivos na altura do diagnóstico. Este estudo durou 24 meses, pelo que a taxa de incidência anual média foi de 268/100 000.
A tuberculose é um problema importante nos PS em Portugal. Considerando as limitações que a PT e IGRA apresentam, a melhor solução parece ser o uso de ambos, utilizando a maior especificidade do IGRA para confirmar uma PT positiva, aproveitando as melhores características de cada teste.

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of positive IGRA results increased with the TST diameter. In those with a TST >15mm, 49.2% were IGRA positive. TST was more than twice as often positive than the IGRA. Therefore, TST+/IGRA- results were more often observed than concordant negative or positive results. In none of the HCWs with a TST+/IGRA- result active TB was diagnosed during the study period. Repeated BCG vaccination increased the number of TST+/IGRA- discordance. The smaller the interval after BCG vaccination, the higher was the TST+/IGRA- discordance.
In the screened HCWs population, active TB was diagnosed in 9. At the time of diagnosis TST and IGRA were positive in all active TB cases. The study period covers 24 months, therefore the average annual incidence rate was 268/100 000.
TB burden in HCWs in Portugal is high. Considering the limitations that TST and IGRA present, the best solution seems to be the use of both, using the IGRA higher specificity for confirming a positive TST, taking advantage of the best characteristics of each test.

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Key-words: Tuberculosis, healthcare workers, tuberculin skin test, interferon-γ release assay, Portugal.

Introduction

The increased risk of healthcare workers (HCWs) for tuberculosis is well established[1-3]. Screening HCWs for latent tuberculosis infection (LTBI) and active tuberculosis (TB) is therefore fundamental in infection control programs in hospitals[4]. For about a century, the tuberculin skin test (TST) has been used to detect LTBI. The TST measures the hypersensitive response to purified protein derivative (PPD), a crude mixture of antigens, many of which are shared by *M. tuberculosis, M. bovis* (source of the Calmette-Guérin bacillus; BCG), and several non-tubercular

mycobacteria (NTM). Although the TST has proved to be useful in clinical practice, it has known limitations, including cross-reactivity with BCG and NTM infections[5]. Advances in molecular biology have led to the development of a new *in vitro* assay that measures interferon (INF)-γ released by sensitized T cells after stimulation with *M. tuberculosis* antigens. These antigens include early secreted antigenic target (ESAT)-6 and culture filtrate protein (CFP)-10. ESAT-6 and CFP-10 are encoded by genes located within the region of difference (RD)-1 segment of the *M. tuberculosis* genome. Two new T-cell-based tests for diagnosing LTBI have been developed and licensed for commercial distribution: QuantiFERON (QFT)-TB Gold in Tube® (Cellestis, Victoria, Australia) uses an enzyme-linked immunosorbent assay (ELISA) to measure antigen-specific production of INF-γ by circulating T cells in whole blood; T-SPOT.TB® (Oxford Immunotec, Oxford, United Kingdom) uses the Elispot technique to measure peripheral blood mononuclear cells that produce INF -γ. These tests are more specific than the TST using PPD, because they use antigens not shared by any of the BCG vaccine strains nor by the more common species of NTM (e.g. *M. avium*)[6]. Research on test performance has shown that assays using RD1 antigens are more specific than TST, have a better correlation with surrogate measures of exposure to *M. tuberculosis* in low incidence settings, and exhibit less cross-reactivity due to BCG vaccination than the TST[7,8]. In the absence of a gold standard for LTBI, active TB is used as a surrogate for LTBI to judge sensitivity. In a meta-analysis, the pooled sensitivity and specificity for the IGRA were higher than for TST[9]. The IGRA has potential advantages besides its greater specificity, including logistical convenience (no second patient contact for reading the test), easier interpretation of the test results (cut-off point is independent from the risk status of the patient) and the ability to perform serial testing without inducing the boosting phenomenon. A recent study demonstrated that the IGRA has merits in screening close contacts for LTBI in low incidence areas[10]. Within the first two years after contact, the progression rate to active TB for IGRA-positive was three times higher than with TST positive contacts.
So far only few systematic investigations of LTBI in HCWs using the IGRA have been published[11-17]. Therefore, we screened for LTBI in Portuguese HCWs using IGRA and TST and compared the performance of boht tests.

Materials and methods

Study setting and study subjects

In compliance with EU regulations, the Hospital S. João, Porto – Portugal, implemented an Occupational Health Division for the hospital staff. Since May 2007 the workers of this hospital have been offered TB screening with TST and IGRA simultaneously by this Division. Screening follows the Centers for Disease Control and Prevention (CDC) guidelines[4]. Upon commencement of employment, all workers are examined to exclude active TB and to assess their pre-employment status. Depending on risk assessment, the examination is repeated annually or every other year. HCWs with close patient contact in the infection and TB wards are considered at high risk, workers with regular patient contact in the

other wards are considered at medium risk and workers with no regular patient contact or no contact with biological material are considered at low risk. Screening is performed annually for those with contact with TB patients or infectious material. For all other HCWs screening is scheduled bi-annually. After unprotected contact with an infectious patient or material, an additional screening is performed as well.

The data presented here comprise all HCWs screened between May 2007 and April 2009 using TST and IGRA simultaneously. TST was only performed when the diameter of a previous TST was below 15 mm or when no previous TST result was known. A chest X-ray was performed in order to exclude active pulmonary disease when TST was 10 mm or higher or IGRA was positive and in HCWs with TB contact or symptoms.

BCG vaccination was assessed through the individual vaccination register. If no register was available, vaccination status was verified by scars. According to the Portuguese National Vaccination Plan[18], BCG vaccination is administered in newborns, and until January 2000 was repeated if TST diameter was below 5 mm. Therefore, every HCW was considered to have been vaccinated at least once.

Tuberculin skin test

TST was performed by trained personnel following standard procedures. Briefly, 0.1 mL (2 TU) of purified protein derivate (PPD, RT23; Statens Serum Institute, Copenhagen, Denmark) were injected in the volar side of the forearm of the participants and read 72 to 96 hours afterwards. The transverse diameter of the induration was measured by experienced personnel.

Before the TST application, the interview was performed and blood for the IGRA was drawn. For the IGRA, the QuantiFERON-TB® Gold In-Tube Assay (Cellestis Limited, Carnegie, Australia) was used. This whole blood assay uses overlapping peptides corresponding to ESAT-6, CFP-10, and a portion of tuberculosis antigen TB7.7 (Rv2654). Stimulation of the antigenic mixture occurs within the tube used to collect blood. Tubes were incubated at 37ºC overnight before centrifugation, and INF-γ release was measured by ELISA following the protocol of the manufacturer. All assays performed met the manufacturer's quality control standards. The test was considered positive when INF-γ was ≥ 0.35 IU after correction for the negative control. Observers were blinded to the results of the TST results.

Statistical analysis

Chi-square test was used to compare frequencies of test results among different groups of participants. For ordered risks, the proportions of positive test results were compared using the chi-square test of trend. The agreement between TST and QFT independent from the agreement by chance alone was assessed by calculating Kappa values for TST >10 mm and TST >15 mm. $P < 0.05$ was considered statistically significant. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated for different putative predictive variables using conditional logistic regression. Model building was performed backwards using the chance criteria for variable selection[19].

Data analysis was performed with SPSS, Version 14 (SPSS Inc., Chicago, Illinois). All people gave their informed consent prior

to their inclusion in the study. No additional data were collected for the study purpose only and analysis was performed with anonymous data. Therefore, no endorsement by an ethics committee was required.

Results

TST and QFT were performed in 1686 HCWs. The population available for analysis comprises 1682 HCWs since QFT was undetermined in 4 (0.2%). Their characteristics are described in Table I. The TST was >10 mm in 78.3% and the QFT was positive in 33.1%. The proportion of positive QFT results increase with the diameter of the TST (Table II). In those with a TST >15 mm, 49.2% are positive in the QFT. Agreement between TST and QFT was higher with >15 mm instead of >10 mm as cut off for TST (0.18 versus 0.29). For both cut offs agreement decreased with number of BCG vaccination (Table III), but even in those vaccinated at birth only, Kappa was low for both the >10 mm (0.26) and for the >15 mm (0.37) cut off for TST. Moving the cut off for TST from >10 to >15 mm did not yield good agreement between QFT and TST (Table III) and as a drawback increased the number of TST-/QFT+ discordant results from 33 (2%) to 202 (12%) as can be calculated from Table II. Therefore, further analysis were undertaken with TST >10 mm as cut off.

With age the proportion of positive TST or QFT increased (Table IV). In those 60 years or older the proportion of positive TST declined compared to those 40-40 years or 50-59 years old while for the QFT a linear association was seen over all age groups. Repeated BCG vaccination was not associated with the probability of a positive TST but decreased the proportion of positive QFT. When a BCG vaccination was performed during the last 10 years, the odds ratio for a positive TST was increased with a borderline statistically significant confidence interval (OR 1.5; 95%CI

Table I – Study population for comparison of IGRA with TST (n=1682)

Age	N	%
16-29 years	601	35.7
30-39 years	463	27.5
40-49 years	313	18.6
50-59 years	271	16.1
≥ 60 years	34	2.0
Gender		
Female	1211	72.0
Male	471	28.0
BCG vaccination		
Only at birth	498	29.6
One additional	631	37.5
Two additional	396	23.5
3-10 additional	157	9.3
Years since last BCG		
> 15 years	1247	74.1
> 10-15 years	277	16.5
1-10 years	158	9.4
Profession		
Administrative	251	14.9
Auxiliaries, cleaning staff	254	15.1
Technicians	119	7.1
Nurses	720	42.8
Physicians	338	20.1
Risk assessment		
Low risk	223	13.3
Moderate risk	626	37.2
High risk	833	49.5
Years working in healthcare		
Start of work	349	20.7
< 5 years	396	23.5
5 ≤ 10 years	263	15.6
10 ≤ 15 years	210	12.5
15-< 20 years	133	7.9
≥ 20 years	331	19.7

Table II – TST diameter by IGRA results

TST	IGRA					
	Negative		Positive		Total	
	N	Row%	N	Row%	N	Col%
0-5 mm	177	94.7	10	5.3	187	11.1
6-10 mm	155	87.1	23	12.9	178	10.6
11-15 mm	423	71.6	168	28.4	591	35.1
> 15 mm	369	50.8	357	49.2	726	43.2
All	1124	66.8	558	33.2	1682	100.0

TST = Tuberculin skin test
IGRA = Interferon-γ-release assay
Row% = Row-percent
Col% = Column-percent

1.0-2.4) and the odds ratio for a positive QFT was well below 1 (OR 0.4; 95%Ci 0.3-0.7). Profession was not associated with the TST, while doctors had a slightly increased odds ratio for a positive QFT (OR 1.4; 95%CI 1.0-2.0), again with a borderline statistically significant confidence interval. Risk assessment, according to the CDC guidelines[4], was neither associated with TST nor with QFT while a positive association was found between years in healthcare and the TST as well as the QFT. Due to colliniarity problems age and years working in healthcare could not be introduced in the same model. Therefore, the effect of these two variables on TST or QFT could not be distinguished.

Table III – Agreement assessed by Kappa between TST and QFT depending on number of BCG vaccinations and cut off for TST

	TST	
	> 10mm	> 15mm
BCG	Kappa (p)	Kappa (p)
at birth	0.25 (0.001)	0.37 (0.001)
1 add	0.16 (0.001)	0.24 (0.001)
2 add	0.11 (0.001)	0.19 (0.001)
3-10 add	0.11 (0.004)	0.17 (0.001)
All	0.18 (0.001)	0.29 (0.001)

p = p value

Most often TST+/QFT– results (47.1%) followed by concordant positive results (31.2%) occurred (Table V). With age the number of concordant positive results increased while the proportion of TST+/QFT– discordant results decreased. Repeated BCG vaccination increased the number of TST+/QFT– discordance. The smaller the interval after BCG vaccination the higher was the proportion of TST+/QFT– discordance. Nurses had less often positive concordant results than administratives, auxiliaries and physicians. At the same time, they had the highest rate of TST+/QFT– discordance. Surprisingly, the higher the assumed risk of infection the lower was the proportion of TST+/QFT+ concordance. Years working in healthcare increased the probability of TST+/QFT+ concordance but were not associated with TST+/QFT– discordance.

In these 1682 HCWs for whom TST and QFT results are available, active TB was diagnosed in 9 HCWs. Diagnosis was based on culture confirmed positive smear in 7 cases, on culture in 1 and on PCR also in 1. At the time of diagnosis TST and QFT were positive in all active TB cases. The study period covers 24 month, therefore the average annual incidence rate was 268/100 000.

Table IV – Proportion of tuberculin skin test (TST) >10 mm and positive interferon-γ-release assay (IGRA) and the respective adjusted odds ratios (OR) and 95% confidence intervals (CI) for different putative risk factors

	TST > 10mm			IGRA-positive		
Age	N (%)	OR	95%CI	N (%)	OR	95% CI
16-29 years	425 (70.7)	1	--	133 (22.1)	1	--
30-39 years	356 (76.9)	1.3	1.0-1.7	150 (32.4)	1.6	1.2-2.1
40-49 years	271 (86.6)	2.5	1.7-3.6	127 (40.6)	1.8	1.3-2.5
50-59 years	238 (87.8)	2.6	1.7-4.0	129 (47.6)	2.0	1.4-2.9
≥ 60 years	27 (79.4)	1.3	0.6-3.0	19 (55.9)	2.6	1.3-5.4
Gender						
Female	951 (78.5)	1	--	390 (32.2)	1	--
Male	366 (77.7)	0.9	0.7-1.2	168 (35.7)	1	0.8-1.2
BCG vaccination						
Only at birth	417 (83.7)	1	--	234 (47.0)	1	--
One additional	487 (77.2)	0.8	0.6-1.1	199 (31.5)	0.7	0.5-0.9
Two additional	296 (74.7)	0.8	0.6-1.2	98 (24.7)	0.7	0.5-0.9
3-10 additional	117 (74.5)	0.9	0.5-1.4	27 (17.2)	0.5	0.3-0.8
Years since last BCG						
> 15 years	996 (79.9)	1	--	479 (38.4)	1	--
> 10-15 years	188 (67.9)	0.7	0.5-0.9	53 (19.1)	0.6	0.4-0.9
1-10 years	133 (84.2)	1.5	1.0-2.4	26 (16.5)	0.4	0.3-0.7
Profession						
Administrative	212 (84.5)	1	--	104 (41.4)	1	--
Auxiliaries, cleaning staff	197 (77.6)	0.8	0.5-1.3	99 (39.0)	1.0	0.7-1.5
Technicians	94 (79.0)	1.0	0.6-1.8	34 (28.6)	0.8	0.5-1.3
Nurses	559 (77.6)	1.2	0.8-1.9	182 (25.3)	0.7	0.5-1.0
Physicians	255 (75.4)	1.0	0.6-1.5	139 (41.1)	1.4	1.0-2.0
Risk assessment						
Low risk	192 (86.1)	1	--	98 (43.9)	1	--
Moderate risk	497 (79.4)	0.9	0.5-1.3	204 (32.6)	0.7	0.5-1.1
High risk	628 (75.4)	0.6	0.4-1.0	256 (30.7)	0.7	0.5-1.0
Years working in healthcare						
Start of work	243 (69.6)	1	--	93 (26.6)	1	--
< 5 years	289 (73.0)	1.3	0.9-1.8	97 (24.5)	1.1	0.8-1.6
5 ≤ 10 years	198 (75.3)	1.3	0.9-1.9	84 (31.9)	1.7	1.1-2.5
10 ≤ 15 years	179 (85.2)	2.5	1.6-4.0	76 (36.2)	2.0	1.3-3.0
15 ≤ 20 years	115 (86.5)	2.7	1.6-4.8	53 (39.8)	2.0	1.2-3.0
≥ 20 years	293 (88.5)	3.1	2.1-4.8	155 (46.8)	1.9	1.3-2.7

Discussion

The study presented here is one of the largest European studies analyzing risk factors for LTBI by using QFT and TST simultaneously. It is also the only cross-sectional study that observed active TB in HCWs in a cross-sectional design. The agreement between TST and QFT was low and could not be improved by increasing the cut off for the TST from >10 to >15 mm because this

Table V – Concordant and discordant tuberculin skin test (TST) >10 mm and interferon-γ-release assay (IGRA) results depending on putative risk factors

	TST/IGRA								
	neg/neg		pos/neg		neg/pos		pos/pos		
Age	N	%	N	%	N	%	N	%	
16-29 years	162	27.0	306	50.9	14	2.3	119	19.8	
30-39 years	98	21.2	215	46.4	9	1.9	141	30.5	<0.001
40-49 years	39	12.5	147	47.0	3	1.0	124	39.6	
50-59 years	28	10.3	114	42.1	5	1.8	124	45.8	
≥ 60 years	5	14.7	10	29.4	2	5.9	17	50.0	
Gender									
Female	236	19.5	585	48.3	24	2.0	366	30.2	0.41
Male	96	20.4	207	43.9	9	1.9	159	33.8	
BCG vaccination									
Only at birth	75	15.1	189	38.0	6	1.2	228	45.8	
Plus one additional	129	20.4	303	48.0	15	2.4	184	29.2	<0.001
Plus two	89	22.5	209	52.8	11	2.8	87	22.0	
Plus 3-10	39	24.8	91	58.0	1	0.6	26	16.6	
Years since last BCG									
> 15 years	225	18.0	543	43.5	26	2.1	453	36.3	
>10-15 years	84	30.3	140	50.5	5	1.8	48	17.3	<0.001
1-10 years	23	14.6	109	69.0	2	1.3	24	15.2	
Profession									
Administrative	33	13.1	114	45.4	6	2.4	98	39.0	
Auxiliaries, cleaning	52	20.5	103	40.6	5	2.0	94	37.0	
Technicians	23	19.3	62	52.1	2	1.7	32	26.9	<0.001
Nurses	151	21.0	387	53.8	10	1.4	172	23.9	
Physicians	73	21.6	126	37.3	10	3.0	129	38.2	
Risk assessment									
Low risk	25	11.2	100	44.8	6	2.7	92	41.3	
Moderate risk	120	19.2	302	48.2	9	1.4	195	31.2	0.001
High risk	187	22.4	390	46.8	18	2.2	238	28.6	
Years in healthcare									
Start of work	94	26.9	162	46.4	12	3.4	81	23.2	
< 5 years	103	26.0	196	49.5	4	1.0	93	23.5	
5 ≤ 10 years	56	21.3	123	46.8	9	3.4	75	28.5	
10 ≤ 15 years	29	13.8	105	50.0	2	1.0	74	35.2	<0.001
15 ≤ 20 years	18	13.5	62	46.6	0	0	53	39.8	
≥ 20 years	32	9.7	144	43.5	6	1.8	149	45.0	
Total	332	19.7	792	47.1	33	2.0	525	31.2	

increased the proportion of TST-/QFT+ results in the whole population from 2% to 12%. TST was more than twice as often positive than the QFT. Therefore, TST+/QFT– results were more often observed than concordant negative or positive results. In none of the HCWs with a TST+/QFT– result active TB was diagnosed during the study period.

So far little is known about the effect of repeated BCG vaccination on the probability of TST+/IGRA– discordant results[20,21]. According to the Portuguese National Vaccination Plan, BCG vaccination is administered in newborns[18]. Therefore, the effect of BCG vaccination on TST and QFT could not be studied. Nevertheless it could be shown that repeated BCG vaccination increased the probability of TST+/QFT– results. When re-vaccination was performed during the last 10 years the chance for discordant TST+/QFT– results was highest (69%) while at the same time probability of concordant positive results was low (15.2%).

Contrary to our preliminary analysis with a smaller sample not only QFT but also TST showed that years working in healthcare are a risk factor for LTBI[22]. Surprisingly, neither risk assessment nor profession was associated with TST or IGRA. In the two European fingerprint studies, the majority of work-related active TB cases occurred when the infection risk was not suspected and preventive measures were not taken[23, 24]. Rotation of the staff is another explanation for the lack of this association, as well as the cross-sectional design of our study, which might dilute the expected association.

The high proportion of TST positive HCWs with repeated BCG vaccination corroborates the conclusion of other authors that TST is not very helpful in populations in which most people are vaccinated and in which vaccination is repeated[25]. In those populations the QFT is a promising alternative even though it is still not clear how the QFT will perform in serial testing. While unlike with the TST[26], the test results cannot be influence by previous QFT, the definition for conversions and reversions of the QFT are not yet well established. Further research will have to elucidate the question about how changes in interferon-γ concentration over time indicate real new infections or clearance of the Mycobacteria can be distinguished from changes in concentration due to natural variation[27,28].

Additionally, and despite our increasing knowledge, several key questions about latent infection and reactivation of *M. tuberculosis* remain unanswered. Particularly, it should be noted that both the TST and the IGRA are designed to identify an adaptive immune response against *M. tuberculosis*, but not necessarily a latent infection. A positive result of currently available diagnostic tests is primarily a measure of an immunological response to stimulation by mycobacterial antigens that should not, therefore, be equated with the presence of live *M. tuberculosis* in the human host. The proportion of individuals who truly remain infected with *M. tuberculosis* after TST or IGRA conversion is unknown. It is also uncertain how long adaptive immune responses towards mycobacterial antigens persist in the absence of live mycobacteria. For these reasons, according to the recently published TBNET consensus statement regarding latent TB, based on the informative value presently derived by IGRA and TST, the term “latent infection” would at best impli-

cate "lasting tuberculosis immune responses" and not necessarily identify a true latent infection with viable microorganisms and potential risk of developing active disease[29,30]. Further studies are needed. Portuguese LTBI treatment guidelines[31], which were revised in 2006, need to be updated, in order to address this question. Whether treatment can be monitored by follow-up of the interferon-γ release is another interesting question to be studied in future[32].

Considering the limitations that both tests present, the best solution seems to be the use of both, using the IGRA higher specificity for confirming a positive TST. This option allows us to take advantage of the best characteristics of each test[30].

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