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Comparison of T-Spot.*TB* and tuberculin skin test among silicotic patients

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Abstract

T-Spot.*TB* and tuberculin skin test were compared in the screening of latent tuberculosis infection among silicotic patients.

A conditional probability model was used to compare the potential clinical utilities of T-Spot.*TB* and tuberculin test performed on 134 silicotic subjects from 1 December 2004 to 31 January 2007. Data from a historical cohort were also reanalyzed for further comparison.

Agreement with T-Spot.*TB* was best using a tuberculin test cutoff of 10mm (kappa=0.432). Age ≥65 independently predicted a tuberculin reaction <10mm (odds ratio: 3.00) but not negative T-Spot.*TB* response. Lower kappa measures were observed among current smokers and those aged ≥65. Tuberculin reaction size was well correlated with both ESAT6 and CFP10 spot counts, except among current smokers. Within the current estimates of sensitivity (88-95%) and specificity (86-99%) for T-Spot.*TB*, the positive likelihood ratio for T-Spot.*TB* test would be substantially higher (6.29-95.0 vs 1.65-1.94) and negative likelihood ratio substantially lower (0.05-0.14 vs 0.32–0.41) than the corresponding ratios for the

tuberculin test. A low TB risk differential was similarly observed between tuberculin-negative and untreated tuberculin-positive subjects in the historical cohort.

T-Spot.*TB* is likely to perform better than tuberculin test in the screening of latent tuberculosis infection among silicotic subjects.

Introduction

Silicotic subjects are at a high risk of developing tuberculosis (TB). Two previous studies in Hong Kong quantified the annual risk of TB in silicotic subjects, which is in the range of approximately 3-5% per annum [1, 2]. Tuberculin skin test (tuberculin test) has been in regular use for the diagnosis of latent tuberculosis infection (LTBI) for many years. A positive reaction can result from infection by *Mycobacterium tuberculosis*, previous Bacillus Calmette Guerin (BCG) vaccination or cross-reaction caused by non-tuberculous mycobacteria [3]. Most of our silicotic patients, with their mean age of 60, were born before large-scale BCG vaccination was introduced in Hong Kong, but little data is available on the impact of other environmental mycobacteria. A cut-off point of 10mm has regularly been employed to diagnose LTBI. The actual sensitivity is unknown because of absence of gold standard. Reduced sensitivity of tuberculin with age has been reported among residents of residential home [4] and elderly patients with culture-confirmed TB [5]. Smoking, alcohol use and body mass index have been found to be independent predictors of a positive tuberculin reaction among silicotic subjects [6], but this could reflect either higher prevalences of LTBI or just underlying differences in the immune status. Interferon-gamma release blood assays have been introduced in recent years. Based on specific antigens identified through genomic research, they are less likely to be

affected by previous BCG vaccination and infections with non-tuberculous mycobacteria [7]. In the absence of a gold standard for LTBI, estimation of diagnostic sensitivity was based primarily on the test-positive rate among patients with culture-confirmed tuberculosis, and estimation of specificity, on the test-negative rate among individuals with low risk of exposure in a low TB incidence area [3, 8]. Preliminary evidences suggest a higher sensitivity and specificity of these tests than the traditional tuberculin test [8-11]. In the targeted screening for LTBI, comparison between these tests has mainly been performed using various measures of agreement like concordance / discordance or kappa measure, with or without correlation with exposure gradient [7-11]. However, the actual significance of such measures is difficult to interpret in day-to-day clinical practice, especially outside the contact settings. We therefore conducted a study comparing an enzyme-linked immunospot assay, T-Spot.*TB* (Oxford Immunotec Ltd, Abingdon, United Kingdom), with tuberculin test in the targeted screening of silicotic subjects, and employed a simple conditional model to examine how measures of agreement could translate into clinically relevant performance characteristics in the target population.

Method

All confirmed silicotic patients with profusion of opacities at category 1 or above, without past history or current suspicion of active TB and not having been offered targeted screening and treatment of latent TB infection, were offered both tuberculin test and T-Spot.*TB* test when they attended the Pneumoconiosis Clinic, the only compensation assessment centre for pneumoconiosis in Hong Kong. Background socio-demographic and clinical information was captured with checking of old BCG scars and measurement of baseline body weight and height. After consent, tuberculin test was done by the Mantoux technique with 2 units of purified protein derivative-RT23 on one of the forearms, and read after 48 to 72 hours. Eight ml of fresh blood was also taken by venipuncture for T-Spot.*TB*, which was conducted and interpreted accordingly to the supplier's protocol as detailed in online supplement E1.

The tuberculin test results as defined by cutoff values of ≥5, 10, and 15mm were compared in turn with the T-Spot.*TB* results for agreement. The effects of socio-demographic and clinical factors on the tuberculin test and T-Spot.*TB* test were also examined.

To facilitate the interpretation of comparison results, a simple deterministic model using conditional probabilities (full description included as online supplement E2) was employed to calculate the sensitivity, specificity, positive likelihood ratio, negative likelihood ratio [12], positive predictive value (PPV) and negative predictive value (NPV) of the T-Spot.TB test and tuberculin test as applied to the study cohort as follows:

1. Assuming the existing surrogate measures of sensitivity and specificity are valid, the following parameters for T-Spot.*TB* test (as based on a recently published meta-analysis [8]) were input into the model --- sensitivity (for adult population): 92% (95% CI: 88% - 95%) and specificity: 92.5% (95%CI: 86% - 99%).
2. The positive and negative likelihood ratios of T-Spot.TB were calculated directly from the assumed sensitivity and specificity as previously described [12].
3. The PPV and NPV of T-Spot.*TB* were calculated through the given assumptions of sensitivity and specificity and the distribution of positive and negative results among the test cohort by solving a set of simultaneous equations as detailed in online supplement E2.

4. The derived PPV and NPV of T-Spot.*TB* were then used as conditional probabilities for a true positive or a true negative for a positive and negative T-Spot.*TB* test respectively for the calculation of the sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, PPV and NPV of the tuberculin test through a two-by-two table of these two tests.

5. The calculations were then repeated for sensitivity analysis, using upper and lower 95% confidence limits of the current estimates of T-Spot.TB sensitivity and specificity.

The positive likelihood ratio reflects the relative likelihood of LTBI among test-positive subjects versus test-negative subjects [12]. In presence of a low proportion (20 – 24 %) of TB cases due to recent transmission in the local population [13,14], it should reflect indirectly the relative risk of developing active TB between test-positive and test-negative subjects. Prospective data comparing the relative risk of active TB between test-positive and test-negative subjects are still pending for the new interferon-release assays [8]. For the tuberculin test, we have recently published a study involving prospective follow-up of a cohort of 435 tuberculin-tested silicotic patients in the same location. Details of the cohort have previously been described [6]. Largely because of patient refusal, a significant proportion of tuberculin-positive

subjects were not treated for LTBI, thus allowing comparison of TB risk between tuberculin-negative and untreated tuberculin-positive subjects during an average follow-up of 5 years. After recategorization of active TB cases found in that cohort by their baseline tuberculin and treatment status, the relative TB risks between different groups were compiled and compared with the predictions of the above model.

Statistical analysis

SPSS version 14 (SPSS Inc, Chicago, Illinois, USA) was used for statistical analysis. In univariate analysis, Chi square test or Fisher exact test were used for unpaired categorical variables (across groups) as appropriate, and McNemar test was used for paired proportions (within same subjects). For numerical variables, ANOVA, Mann-Whitney test and Wilcoxon signed ranks test were used as appropriate. Kappa measure and Spearman's rank correlation were used to assess agreement between tests and correlation of numerical test readings respectively. Multiple logistic regression analysis was employed to control for confounding background / disease variables associated with the test outcome variables ($P<0.20$) in univariate analysis. A two-tailed p value of < 0.05 was taken as statistically significant.

Approval for the study was obtained from the Ethics Committee of the Department of Health of Hong Kong SAR, China. The T-Spot.*TB* test kits were supplied at a reduced price by Oxford Immunotec Ltd.

Results

From 1 December, 2004 to 31 January, 2007, 134 patients underwent both T-Spot.*TB* test and tuberculin test with an uptake rate of about 50% among patients offered the tests in the course of their clinic attendance. Only 6 subjects (4.3%) showed an indeterminate T-Spot.*TB* test because of low cell counts after separation of the mononuclear cells. Repeat blood testing all yielded a determinate result. There were no significant differences in age, presence of comorbidity, and final result between those requiring and not requiring repeat test (Two tailed exact test, all P>0.05). Their background characteristics, as stratified by age (<65 versus ≥65), are summarized in Table 1. Only 2 subjects showed a BCG scar. Both were aged under 65. One of them showed a tuberculin reaction of 7mm and a positive T-Spot.*TB* result. The other one had a tuberculin reaction of 12mm but a negative T-Spot.*TB* result. There was no significant difference in the proportion of subjects with a tuberculin reaction ≥10mm and a positive T-Spot.*TB* test (68.7% vs 64.2%, McNemar test, p = 0.391). A higher proportion of subjects aged under 65 had a positive tuberculin reaction (≥10mm) as compared to those aged 65 or above (p=0.01), but no significant association was observed between T-Spot.*TB* test status and age (Table 1).

Table 2 summarizes the univariate analysis of the background characteristics according to tuberculin test and T-Spot.*TB* status. Older age was associated with a tuberculin reaction <10mm, but no significant association was found between T-Spot.*TB* test and any of the characteristics listed in Table 2. Higher proportions of current smokers than never / ex-smokers were found to have tuberculin reaction ≥10mm (81.3% vs 64.7%, p=0.078) and positive T-Spot.*TB* test (78.1% vs 59.3%, p=0.059), but the differences just failed to reach statistical significance. On multiple logistic regression analysis using predictor variables associated with test outcomes (P<0.20) in univariate analysis (Table2), age ≥65 remained an independent predictor of a negative tuberculin reaction < 10mm (OR: 3.00, 95%CI:1.38 - 6.55, P=0.006) but not a negative T-Spot.*TB* response (OR: 1.57, 95%CI:0.74 - 3.55, P=0.636), while current smoking did not significantly predict a positive tuberculin response ≥10mm (O.R. 2.10, 95%CI: 0.77-5.82, p=0.146) or a positive T-Spot.*TB* test (O.R. 2.27, 95%CI: 0.89-5.78, p=0.085).

The median (lower- upper quartile) tuberculin reaction sizes were 0 (0.0 - 6.3) mm and 16 (14 - 20) mm for those with a tuberculin reaction size <10mm and ≥ 10mm respectively. Of the 86 subjects with a positive T-Spot.*TB* test, 29 (33.7%) had a net spot count (peptides-well count – control well count) of 6 or more for the ESAT6

(early secretary antigenic target 6) peptides well alone, 18 (20.9%) for the CFP10 (culture filtrate protein 10) peptides well alone, and 39 (45.3%) both. The median (inter-quartile range) net spot counts for the ESAT6 peptides well and CFP10 peptides well were 16.5 (6.0 - 32.0) and 10.5 (2.8 - 52.0) respectively (Wilcoxon Signed Ranks Test, p =0.830). Higher tuberculin reaction sizes (median 15.0 mm vs 10.0 mm, Mann-Whitney test, p=0.001), but not net ESAT6 well spot counts (median 6.0 vs 4.0, Mann-Whitney test, p=0.920) or CFP10 well spot counts (median 3.0 vs 2.0, Mann-Whitney test, p=0.300), were observed among those aged < 65 than those aged 65 or above. Higher tuberculin reaction sizes (median 16.0 mm vs 12.5 mm, Mann-Whitney test, p=0.016) and net ESAT6 well spot counts (median 15.5 vs 4.0, Mann-Whitney test, p=0.013), but not CFP10 well spot counts (median 4.0 vs 3.0, Mann-Whitney test, p=0.243), were observed among current smokers than never / ex-smokers. Table 3 summarizes the correlation between tuberculin reaction size and the net spot counts for the ESAT6 and CFP10 peptides wells among the whole cohort before and after stratification by age and smoking.

Table 4 summarized the comparison of T-Spot.*TB* with the tuberculin test using different cut-off points. The concordance rates between T-Spot.*TB* and the tuberculin test were 71.6%, 74.6%, and 67.9% for the cutoff points of 5mm, 10mm, and 15mm

respectively. Higher concordance rates were found among T-Spot.*TB* test-positive subjects than T-Spot.*TB* test-negative subjects for the tuberculin cutoff points of 5mm (89.5% vs 39.6%, $p<0.001$) and 10mm (83.7% vs 58.3% $p=0.001$), but the opposite was true for the 15mm cutoff (35.8% vs 64.2%, $p=0.037$). Overall and for all subgroups (age < 65, age ≥65, current smokers and never / ex-smokers), the kappa measure for agreement between T-Spot.*TB* and the tuberculin test was at a maximum with a cut-off reaction size of 10mm for the latter. For all cut-offs, the kappa measures of agreement between tuberculin test and T-Spot.*TB* test were similar but greater for the younger age group than the older age group. Much lower kappa measures were observed for current smokers in contrast with those for never / ex-smokers.

Table 5 summarizes the estimated sensitivity, specificity, positive and negative likelihood ratios, and positive and negative predictive values of T-Spot.*TB* and tuberculin test (cutoff =10mm) for the study cohort under the current estimates of T-Spot.*TB* sensitivity (88-95% for adult population) and specificity (86-99%) [8]. Substantial differences in test characteristics were observed between T-Spot.*TB* and tuberculin test across the full range of assumptions. The estimated sensitivity of tuberculin test varied from 78.7 to 81.6% and specificity from 52.4 to 57.9%. The

positive likelihood ratio for T-Spot.*TB* test was higher (6.29-95.0 vs 1.65-1.94) and negative likelihood ratio lower (0.05–0.14 vs 0.32–0.41) than the corresponding ratios of the tuberculin test.

From a separate historical cohort of 435 silicotic patients tuberculin-tested between Aug 1, 1995 and Dec 31, 2002 [5], 17 (14.4%) out of 118 patients with tuberculin reaction <10mm, 45 (20.8%) out of 216 untreated patients with tuberculin reaction ≥10mm, and 11 (10.9%) of 101 patients with tuberculin reaction ≥10mm and treated for LTBI (with either 6 months of isoniazid or two months of rifampicin and pyrazinamide at a treatment completion rate of 70%) developed active TB after 5.2 +/- 2.3 (mean +/-SD) years of follow-up till the end of 2005. The corresponding TB incidences rates were 2596 (tuberculin-negative subjects), 3778 (untreated tuberculin-positive subjects) and 2582 (treated tuberculin-positive subjects) per 100000 person-years respectively. The TB incidence ratios were observed to be 0.69 (2596 / 3778) between tuberculin-negative and untreated tuberculin-positive subjects, and 0.68 (2582 / 3778) between treated and untreated tuberculin-positive subjects respectively. The relatively low risk differential between tuberculin-negative and untreated tuberculin-positive subjects was generally in line with the low positive

likelihood ratios of tuberculin test as estimated from the conditional probability model (Table 5).

Discussion

In this study, moderate agreement was found between T-Spot.*TB* and tuberculin test using 10mm as cut-off point among a high risk cohort of silicotic subjects in Hong Kong (Table 4). However, older age was significantly associated with a negative tuberculin test response while no such associations were observed for T-Spot.*TB* (Table 2). There was also a trend towards association between current smokers and a positive response in both tests. Poorer agreement was observed between T-Spot.*TB* and tuberculin test among older subjects and current smokers (Table 4). Dissociation of T-Spot.*TB* spot counts from the tuberculin reaction size was also observed among current smokers (Table 3). Within the current estimates of its sensitivity and specificity [8], the T-Spot.*TB* test would be expected to perform significantly better than the tuberculin test (Table 5).

A lower sensitivity of tuberculin test among elderly subjects is well reported in the literature [4, 5, 15]. In this study, the lower percentage of tuberculin-positive subjects among those aged 65 or above likely reflected lower test sensitivity, rather than lower prevalence of LTBI. Among this fully ambulant cohort, no significant difference in co-morbidities (other than silicosis and hypertension) was observed between

tuberculin-positive and tuberculin-negative subjects (Table 2), despite the higher prevalence of these co-morbidities among the elderly. Age therefore appeared to affect the interpretation of tuberculin test even in absence of major co-morbidities. On the other hand, T-Spot.*TB* was not significantly affected by age, and this could support its role in the targeted screening of LTBI among elderly subjects.

The association between smoking and a positive tuberculin reaction is also well reported in the literature [6, 16, 17]. However, in contrast with the effect of age, it is usually assumed to indicate a higher LTBI prevalence, likely as a result of increased exposure. In this study, significantly higher tuberculin reaction sizes and net ESAT6 well counts were observed among current smokers, in contrast with never / ex-smokers. It is noteworthy that the association of smoking and tuberculin-positivity as observed in this and an earlier silicotic cohort [6] was seen predominantly among current smokers, with ex-smokers at much lower risk and the total number of cigarette pack-years did not increase the risk of a positive response (Table 2). The hypothesis of increased exposure among smokers (and hence higher true prevalence of LTBI) might not be adequate to account for such observations. As smoking has been well associated with inflammatory responses within the lungs [18-20], there remains a possibility that it could boost reactivity to tuberculin and the more specific antigens.

The dissociation of the tuberculin reaction size from the T-Spot.*TB* spot counts among current smokers could also suggest that the magnitude of such boosting differs for different antigens.

The agreement between T-Spot.*TB* and tuberculin test was at a maximum using a cut-off of 10mm (Table 4), supporting our previous use of such cut-off among the silicotic subjects, despite the recommendation of a lower cutoff of 5mm by some authorities [3]. However, the agreement between the T-Spot.*TB* test and tuberculin test of 75.9% (kappa=0.474) was somewhat lower than that previously reported for BCG-unvaccinated immunocompetent subjects in another study [11]. A higher mean age has likely contributed to such observation, in line with the lower agreement observed among those aged 65 or above.

As shown in Table 5, under the deterministic conditional model and the ranges of T-Spot.*TB* sensitivity and specificity derived from a recent meta-analysis, the tuberculin test would have a sensitivity of 78.7-81.6%, which was in reasonable agreement with the estimated tuberculin sensitivity (68-78% for adult population) in the same meta-analysis [8]. However, the specificity of 52.4-57.9% was exceptionally low for a group with very low BCG vaccination coverage [8]. Cross-reactivity from

previous exposure to non-tuberculous mycobacteria could be a possible explanation [3], but differing abilities between T-Spot.TB and tuberculin test in detecting recent and remote infection might well be an alternative explanation [21-22]. Overall a very low discriminating power of the tuberculin test was predicted, with a positive likelihood ratio consistently lower than two and a negative likelihood ratio of around one-third (table 5). A relative risk of 0.69 was actually observed between tuberculin-negative and untreated tuberculin-positive subjects in a similar historical cohort. The residual risk among those screened negative was over 2500/100,000 person-years. New infection or reinfection after tuberculin testing cannot account for the high residual risk, as previous molecular analysis of culture isolates showed only a low percentage of clustering, suggesting a low proportion of disease (20-24%) due to recent transmission in Hong Kong [13-14].

Silicotic patients in high TB prevalence areas are a well-known high risk group for targeted screening of LTBI. Although the study was based on a convenient sample of silicotic patients attending a compensation assessment centre, this is the usual clinical setting where such targeted screening is offered. The absence of a gold standard remains a major problem in the comparison of diagnostic tests for LTBI. Similar to other studies [7-11, 21-24], no direct information was provided as to how well the

new interferon-gamma release assay was able to predict subsequent risk of active TB disease. Indirect estimates of sensitivity and specificity through surrogate measures might not reflect actual test performance for the latent infection state. Although the specific antigens employed in the new test might help to reduce interference by cross-reactivity, insufficient information is available as to their ability to differentiate between recent and remote infections. However, the derived sensitivity of tuberculin test was well in keeping with previous estimates from surrogate measures. The limited discriminating power of the tuberculin test under the model was also supported by actual observation in a similar historical cohort in the same location, even though it must be admitted that the two cohorts were not identical [6]. Notwithstanding all the potential limitations, this study highlighted potentially major differences in performance between the T-Spot.*TB* test and the tuberculin test among silicotic patients in a high TB prevalence setting. Further studies are indicated to identify an optimal strategy for targeted screening of LTBI in such situation.

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Table 1: Distribution of background characteristics, tuberculin status and T-Spot.*TB* results as stratified by age < 65 and ≥65

Variables	Overall N=134	<65 years N=91	≥65 years N=43	P`
Male Sex	97	98	95	0.59†
Smoking Status				0.33
Never	17.2	15.4	20	
Ex-smoker	59	57	62	
Current	23	27	16.3	
Regular Alcohol Use	10.4	11.0	9.3	1.00†
BCG scar	1.5	2.2	0.0	1.00†
With comorbidity‡	18.7	9.9	37	<0.001
Principal Job				<0.01
Und. Driller[a]	44	54	20	
Surf. Driller[b]	37	30	51	
Other	18.7	14.3	28	
Nodule Profusion				0.49
Category 1	72	74	70	
Category 2	26	24	30	
Category 3	1.5	2.2	0	
Nodule Size				0.94
<1.5 mm	26	25	28	
1.5-3 mm	65	66	63	
3-10 mm	9.0	8.8	9.3	
PMF#	19.4	16.5	26	0.21
Tuberculin Reaction ≥10mm	69	77	51	0.01
Positive T-Spot.*TB* test	64	68	56	0.17
Age*, years	60.4+/-9.7	54.8+/-5.3	72.2+/-5.3	-
Cigarette pack-years*	21. 4+/-20.3	17.3+/-16.7	30.1+/-24.4	<0.001
Body Mass Index*	24.2+/-3.0	24.0+/-3.0	24.5+/-3.2	0.45
Dust exposure*, yr	22.9+/-8.7	20.4+/-7.2	28.2+/-9.3	<0.001

Figures presented are percentages unless stated otherwise.

* mean+/-SD

#Progressive Massive Fibrosis

† Fisher Exact, two-tailed P value

‡ Diabetes mellitus, heart / cerebrovascular disease, malignancies, cirrhosis, renal and gastrointestinal diseases; hypertension alone was not included

[a] Underground drillers: caisson workers / tunnel workers / miners

[b] Surface drillers / stone-splitters / drillers in construction trade or quarries

Table 2: Distribution of background characteristics as stratified by tuberculin test and T-Spot.*TB* status

Variables	Tuberculin Reaction			T-Spot.*TB*		
	<10mm N=45	≥10mm N=88	P`	Negative N=49	Positive N=84	P`
Male Sex	98	97	1.00†	100	95	0.30†
Smoking Status			0.19			0.14
Never	21	15.2		16.7	17.4	
Ex-smoker	64	57		69	54	
Current	14.3	28		14.6	29	
Alcohol Use	14.3	8.7	0.33	12.5	9.3	0.56
BCG scar	2.4	1.1	0.53†	2.1	1.2	1.00†
Comorbidity‡	16.7	19.6	0.69	18.8	18.6	0.98
Principal Job			0.50			0.31
Und. Driller[a]	38	47		35	49	
Surf. Driller[b]	38	37		42	35	
Other	23	16.3		23	16.3	
Nodule Profusion			0. 76			0.56
Category 1	69	74		73	72	
Category 2	29	25		27	26	
Category 3	2.4	1.1		0.0	2.3	
Nodule Size			0.88			0.52
<1.5 mm	26	26		27	25	
1.5-3 mm	67	64		60	67	
3-10 mm	7.1	9.8		12.5	7.0	
PMF#	23.8	17.4	0.38	25	16.3	0.22
Age*, years	63.6+/-9.0	58.9+/-9.7	0.01	61.3+/-9.8	59.9+/-9.7	0.42
Cigarette pack-yr*	24.7+/-24.6	19.9+/-18.0	0.20	24.8+/-20.2	19.5+/-18.8	0.28
Body Mass Index*	23.8+/-3.5	24. 3+/-2.8	0. 35	24.0+/-3.5	24.4+/-2.7	0.45
Dust exposure*, yr	23.2+/-9.7	22.8+/-8.3	0.77	23.8+/-7.6	22.4+/-9.3	0.39

Figures presented are percentages unless stated otherwise.

* mean+/-SD

#Progressive Massive Fibrosis

† Fisher Exact, two-tailed P value

‡ Diabetes mellitus, heart / cerebrovascular disease, malignancies, cirrhosis, renal and gastrointestinal diseases; hypertension alone was not included

[a] Underground drillers: caisson workers / tunnel workers / miners

[b] Surface drillers / stone-splitters / drillers in construction trade or quarries

Table 3: Correlation between tuberculin reaction size and the net spot counts in T-Spot.*TB* test among the whole cohort before and after stratification by age and smoking

Group	N	ESAT6 well		CFP10 well	
		Correlation*	P	Correlation*	P
Whole cohort	134	0.336	<0.001	0.243	<0.01
Age < 65	91	0.324	<0.01	0.253	0.02
Age ≥ 65	43	0.422	<0.01	0.197	0.21
Current smokers	32	0.030	0.87	-0.096	0.60
Never / Ex-smokers	102	0.375	<0.001	0.308	<0.001

*Spearman rank correlation coefficients between the tuberculin reaction size and the net spot count (test well count – negative control well count) of T-Spot.*TB* test

Table 4: Comparison of T-Spot.*TB* with tuberculin test using different cut-off points

Tuberculin Test	T-Spot.*TB* All	Negative	Positive	Concordance	kappa	P
Whole Cohort						
<5mm	28(100)	19(67.9)	9 (32.1)		-	-
≥5mm	106(100)	29(27.4)	77(72.6)	71.6	0.321	<0.001
<10mm	42(100)	28(66.7)	14(33.3)			
≥10mm	92(100)	20(21.7)	72(78.3)	74.6	0.432	<0.001
<15mm	71(100)	38(53.5)	33(46.5)			
≥15mm	63(100)	10(15.9)	53(84.1)	67.9	0.369	<0.001
Age <65						
Overall	91(100)	29(31.9)	62(68.1)		-	-
≥5mm	78(100)	19(24.4)	59(75.6)	75.8	0.347	<0.001
≥10mm	70(100)	14(20.0)	56(80.0)	78.0	0.454	<0.001
≥15mm	52(100)	8(15.4)	44(84.6)	71.4	0.397	<0.001
Age ≥65						
Overall	43(100)	19(44.2)	24(55.8)		-	-
≥5mm	28(100)	10(35.7)	18(64.3)	62.8	0.229	0.13
≥10mm	22(100)	6(27.3)	16(72.7)	67.4	0.347	0.02
≥15mm	11(100)	2(18.2)	9(81.8)	60.5	0.252	0.04
Current Smokers						
Overall	32(100)	7(21.9)	25(78.1)		-	-
≥5mm	29(100)	7(24.1)	22(75.9)	68.8	-0.151	0.34
≥10mm	26(100)	5(19.2)	21(80.8)	71.9	0.133	0.45
≥15mm	22(100)	4(18.2)	18(81.8)	65.6	0.129	0.45
Never/ Ex-Smokers						
Overall	102(100)	41(40.2)	61(59.8)		-	-
≥5mm	77(100)	22(28.6)	55(71.4)	72.5	0.390	<0.001
≥10mm	66(100)	15(22.7)	51(77.3)	75.5	0.480	<0.001
≥15mm	41(100)	6(14.6)	35(85.4)	68.6	0.396	<0.001

Percentages quoted in ( ) are percentages by rows.

Table 5: Deterministic model prediction on test performance of T-Spot.*TB* and tuberculin test for the study cohort under different assumptions of T-Spot.*TB* sensitivity and specificity

T-Spot.*TB*						Tuberculin test (cutoff=10mm)					
Sen*	Sp*	LR+	LR-	PPV	NPV	Sen	Sp	LR+	LR-	PPV	NPV
92.0%	92.5%	12.27	0.09	96.2%	85.0%	80.4%	55.2%	1.79	0.36	78.5%	58.0%
92.0%	99.0%	92.00	0.08	99.5%	84.5%	80.4%	57.9%	1.91	0.34	81.3%	56.5%
92.0%	86.0%	6.57	0.09	92.2%	85.6%	80.4%	52.4%	1.69	0.37	75.3%	59.7%
95.0%	92.5%	12.67	0.05	95.9%	91.0%	81.6%	55.2%	1.82	0.33	77.0%	62.0%
95.0%	99.0%	95.00	0.05	99.5%	90.6%	81.6%	57.9%	1.94	0.32	79.9%	60.6%
95.0%	86.0%	6.79	0.06	91.7%	91.4%	81.6%	52.4%	1.72	0.35	73.6%	63.7%
88.0%	92.5%	11.73	0.13	96.5%	76.4%	78.7%	55.2%	1.76	0.39	80.7%	52.1%
88.0%	99.0%	88.00	0.12	99.6%	75.7%	78.7%	57.9%	1.87	0.37	83.2%	50.6%
88.0%	86.0%	6.29	0.14	93.0%	77.3%	78.7%	52.4%	1.65	0.41	77.7%	53.9%

Sen: Sensitivity = number of test positives / number of all true positives

Spec: Specificity = number of test negatives / number of all true negatives

LR+: Positive Likelihood Ratio =sensitivity / (1 – specificity)

LR-: Negative Likelihood Ratio =(1-sensitivity) / specificity

PPV: Positive Predictive Value = number of true positives / number of test positives

NPV: Negative Predictive Value = number of true negatives / number of test negatives

* Model assumptions based on the best estimates and upper and lower 95% confidence levels of the sensitivity and specificity of T-Spot.*TB* test as derived from a recently published meta-analysis [8]