ORIGINAL ARTICLE

Effect of immunosuppressive therapy on interferon γ release assay for latent tuberculosis screening in patients with autoimmune diseases: a systematic review and meta-analysis

Sunny H Wong,[1,2] Qinyan Gao,[1,3] Kelvin K F Tsoi,[4] William K K Wu,[5] Lai-shan Tam,[1] Nelson Lee,[1] Francis K L Chan,[1,2] Justin C Y Wu,[1,2] Joseph J Y Sung,[1,2] Siew C Ng[1,2]

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For numbered affiliations see end of article.

Correspondence to
Dr Siew C Ng, Department of Medicine and Therapeutics, 30–32 Ngan Shing Street, Prince of Wales Hospital, The Chinese University of Hong Kong, 9/F Lui Che Woo Clinical Sciences Building, Shatin, Hong Kong; siewchienng@cuhk.edu.hk

SHW and QG contributed equally.

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ABSTRACT

Objective Interferon γ release assay (IGRA) is commonly used to diagnose latent TB infection (LTBI). Immunosuppressive therapy may affect its performance but data are conflicting. We aimed to determine the effect of immunosuppressive therapy on the performance of IGRA in patients with autoimmune diseases.

Methods We searched PubMed, MEDLINE, EMBASE and the Cochrane Library up to December 2014. We included studies that reported the IGRA results in patients with autoimmune disease with or without immunosuppressive therapy. The pooled effect of immunosuppressive therapy on IGRA was estimated using a Peto fixed-effects model.

Results We included 17 studies with 3197 participants in the meta-analysis. Among the subjects, 71.5% were taking immunosuppressive therapy and 56.7% had received Bacillus Calmette–Guérin vaccination. Compared with patients not on immunosuppressants, patients receiving immunosuppressive therapy were less likely to have a positive IGRA result (OR 0.66, 95% CI 0.53 to 0.83, I^2=23%), especially patients receiving anti-tumour necrosis factor (anti-TNF) treatment (OR 0.50, 95% CI 0.29 to 0.88). The use of immunosuppressive therapy was also associated with a lower rate of positive tuberculin skin test result (OR 0.51, 95% CI 0.42 to 0.61).

Conclusions Our meta-analysis showed that IGRA results are negatively affected by immunosuppressive therapy. IGRA alone may not be sufficiently sensitive to diagnose LTBI in patients on immunosuppressive therapy. Patients should preferably be screened for LTBI before initiation of immunosuppressive therapy, especially before anti-TNF therapy.

Key messages

What is the key question?

- What is the performance of interferon γ release assay (IGRA) in diagnosing latent TB infection (LTBI) in patients on immunosuppressive therapy?

What is the bottom line?

- Screening and treating for LTBI can reduce the rate of TB reactivation and help control the disease globally; however, there are conflicting data on the performance of IGRA in immunocompromised individuals.

Why read on?

- This meta-analysis shows that IGRA results are negatively affected by immunosuppressive therapy, thus IGRA alone may not be sufficient to diagnose LTBI in patients on immunosuppressive therapy.

INTRODUCTION

TB is a major infectious disease. It was estimated that one-third of the world's population had latent TB infection (LTBI),[1] of which 5–10% would develop active TB disease if not being treated. Identifying LTBI is therefore important to control the disease globally. Recently, interferon γ release assay (IGRA) has provided an alternative method to tuberculin skin test (TST) in diagnosing LTBI. As IGRA measures interferon γ release by T cells after stimulation with specific TB antigens, it does not cross react with Bacillus Calmette–Guérin (BCG) and is free from false-positive results in vaccinated individuals.[2] It has been shown to have a superior sensitivity and specificity than TST in the general population.[3] The commercially available QuantiFERON-TB Gold In-Tube test (QFT) and T-SPOT.TB test (T-SPOT) have been approved for all situations in which TST is indicated.[4]

Despite its performance in otherwise healthy individuals, there are limited data on the performance of IGRA in patients who are immunocompromised. This clinical uncertainty is recognised by WHO and the US Centers for Disease Control and Prevention (CDC).[4] Currently, the National Institute for Health and Care Excellence (NICE) recommends either an IGRA alone or an IGRA with a concurrent TST in diagnosing LTBI in people who are immunosuppressed.[5] Immunosuppressive therapy has been increasingly used for a wide range of autoimmune disorders, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel diseases (IBD) that collectively affect 5–9% of the western population.[6] Many of these patients are receiving corticosteroids, immunosuppressive drugs or monoclonal antibodies such as anti-tumour necrosis factor (anti-TNF) agents. These medications

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are associated with a heightened risk of infection, including reactivation of LTBI and other opportunistic infections.[7] Testing for LTBI has become mandatory before commencing potent immunosuppressive therapy such as anti-TNF in these patients.

Although a number of studies have assessed the performance of IGRA in patients with autoimmune diseases, uncertainty remains regarding the effect of immunosuppressive therapy on IGRA. Many immunosuppressive agents are potent inhibitors of T cells and may impair the interferon-γ response.[8] Several studies have reported lower positive or higher indeterminate rates among patients receiving immunosuppressive therapy, although considerable variability existed between studies. Over the next decade, the number of immunosuppressive and biologic agents will continue to expand. We will be caring for an ever aging population with autoimmune diseases and other complex morbidities; therefore the accurate diagnosis of LTBI has become even more important. In this systematic review and meta-analysis, we investigated the effect of immunosuppressive therapy on IGRA in patients with autoimmune diseases.

METHODS

Data sources and searches

This study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[9] An electronic literature search was conducted of articles using the following keywords alone and in combination: 'interferon gamma release assay', 'interferon gamma assay', 'IGRA', 'QuantiFERON', 'T-SPOT.TB', 'tuberculosis', 'autoimmune diseases', 'immune mediated inflammatory diseases', 'inflammatory diseases', 'inflammatory bowel diseases', 'Crohn's disease', 'ulcerative colitis', 'rheumatic diseases', 'rheumatological diseases', 'connective tissue diseases', 'psoriasis', 'psoriatic arthritis', 'rheumatoid arthritis', 'ankylosing spondylitis', 'spondyloarthritis', 'systemic lupus erythematosus' and 'lupus' to identify clinical studies in full publications from six computerised databases: PubMed (1966 to Dec 2014), MEDLINE (1948 to Dec 2014), EMBASE (1974 to Dec 2014), and six databases within the Cochrane Library (1991 to Dec 2014). Only publications in English were included. After removal of duplicate references, initial screening of article titles and abstracts was undertaken by two independent members (QYG and SHW). This process removed articles that were not relevant to our study, including editorials, case reports and therapeutic approach articles. Potentially relevant articles were obtained in full text and reviewed independently. Predefined criteria were used to determine eligibility for inclusion. Disagreement at any stage between the two reviewers was resolved by consensus; if consensus could not be reached, a third reviewer (SCN) was consulted for a final decision. We obtained copies of all articles identified as being of potential importance.

Study selection

Clinical studies were eligible for this meta-analysis if they met the following criteria: (1) clinical studies that assessed the performance of an IGRA in autoimmune diseases; (2) original counts of the results were provided or could be calculated for individuals with and without immunosuppressive therapy. Studies were excluded if they: (1) were not written in English; (2) evaluated a non-commercial, in-house or old generation IGRA; (3) reported insufficient data on IGRA results or had an inappropriate study design that did not assess the performance of an IGRA; (4) were review articles or commentaries. Moreover, if a study provided results for both QFT and T-SPOT, the test with a lower indeterminate rate was used in the combined analysis.

Data extraction and quality assessment

Data were extracted and assessed for eligibility by two independent reviewers. Extracted data included the primary author, year of publication, country of origin, study design and setting, study size, BCG vaccination rate, proportion of patients on immunosuppressive therapy, disease type, therapeutic medication, methods of IGRA, and IGRA results (positive, negative or indeterminate) with and without immunosuppressive therapy. If TST results were available, the counts of the results were also recorded. As the studies were conducted in different countries with different TB incidence and BCG vaccination rates, we accepted the definition of a positive TST result used by individual studies.

The main outcome measure was the effect of immunosuppressive therapy on the positive rate of IGRA in patients with autoimmune disease. Secondary outcomes included its effect on the indeterminate rate of IGRA and the positive rate of TST. Subgroup analyses were performed for different methods of IGRA, different medication or disease types.

The quality of included studies was evaluated by using a modified version of QUADAS quality assessment tool which is recommended in systematic reviews of diagnostic accuracy studies.[10] Given the lack of a reference gold standard in the diagnosis of LTBI, modification of QUADAS was warranted. Each QUADAS item was scored as 'yes', 'no' or 'unclear.' Two reviewers (QYG and SHW) independently reviewed all QUADAS items for each included study with disagreements being resolved by consensus.

Data synthesis and analysis

The results from included studies were analysed using Review Manager V.5.1 (Nordic Cochrane Centre, Denmark). OR with 95% CI was used to evaluate the test results among patients with autoimmune disease with or without immunosuppressive therapy. The null hypothesis was rejected at a statistical significance level of $p<0.05$. Weighted summaries were determined using meta-analysis models if a given result was reported by four or more studies. Tests for heterogeneity were performed for each meta-analysis using Cochrane's Q test and I^2 statistic ($I^2<25\%$ or $>50\%$ reflects small or large inconsistency respectively). If the heterogeneity test was not significant ($p>0.1$), the Peto fixed-effects model was used because positive events were not particularly common in some studies; otherwise the DerSimonian and Laird random effects model would be performed.

RESULTS

Description of the included studies

The initial search identified 372 abstracts (figure 1). The majority of abstracts were excluded as they were not relevant to the search topic. A total of 83 English language abstracts which involved human subjects were retrieved. Following exclusion, 17 studies comprising 3197 patients published between 2008 and 2014 were included in the final analysis.[11–27] The study characteristics are shown in table 1.

Most of the studies were conducted in Europe, apart from one conducted in Hong Kong[26] and two studies conducted within the Asian border of Turkey.[17 27] The mean age of the subjects was 44.9±4.7 years. There was a fair gender distribution (55.6% female), except in two SLE studies which included predominantly female participants.[12 27] The number of subjects

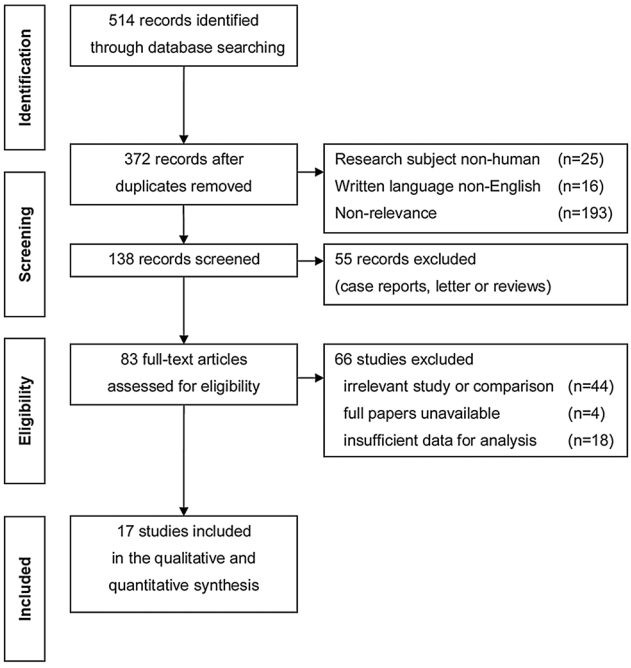

Figure 1 Summary of database search and paper selection.

in each study ranged from 50 to 563. The average BCG vaccination rate was 56.7% with a range from 1.1% in an Italian study[11] to 100% in an Austrian study.[23] The mean percentage of subjects on immunosuppressive therapy was 71.5%, ranging from 43.0% to 88.7% (table 2).[18 20] The majority of the studies evaluated QFT except for three studies which tested T-SPOT[12 16 19]; three other studies evaluated QFT and T-SPOT.[13 20 21] The indeterminate rates of IGRA ranged from 1.1%[11] to 17.6%.[25] TST was performed in all of the studies. The mean concordance rate between TST and IGRA was 77.1%, ranging from 53.3% to 89.2%.[17 18] All studies were published as full papers.

Study quality

The QUADAS assessment tool was used to assess the quality of the studies. Overall, most studies satisfied the modified QUADAS items for quality analysis (see online supplementary table S1). However, we observed a high degree of uncertainty for QUADAS items 2 and 4, as many studies did not describe the selection criteria in details and did not mention if IGRA was performed prior to TST. Four of the 17 included papers did not report the indeterminate test results.[17–19 24]

Effect of immunosuppressive therapy on IGRA in autoimmune diseases

The pooled estimate of 3197 individuals with autoimmune diseases showed that patients on immunosuppressive therapy were less likely to have a positive IGRA result compared with those not receiving immunosuppressive therapy (OR 0.66, 95% CI 0.53 to 0.83). There was no significant heterogeneity of effects between studies. The plots are shown in figure 2 and online supplementary figure S1. This suggested immunosuppressive therapy may negatively affect the IGRA results. Subgroup analyses showed a significant negative impact on the QFT results (OR 0.65, 95% CI 0.50 to 0.84) (figure 2).[11 13–15 17 18 20–27] A consistent trend was also observed for T-SPOT; though the pooled estimate from the six studies did not reach statistical significance (OR 0.81, 95% CI 0.59 to 1.10) (figure 2).[12 13 16 19–21] Nine studies assessed the effect of immunosuppressive therapy on the indeterminate IGRA results.[11–13 15 16 23 25–27] The pooled estimate showed no significant association between immunosuppressive therapy and the rate of indeterminate results in IGRA (OR 1.10, 95% CI 0.75 to 1.61) (see online supplementary figure S2).

Effects of different types of immunosuppressive therapy

We further evaluated the effects of different immunosuppressive therapies on the IGRA results. Commonly used therapeutic regimens in autoimmune diseases included steroids, oral immunosuppressants and anti-TNF therapy. We identified 11 studies that provided IGRA results in patients taking steroids.[12–16 18 20 22 23 25 26] The pooled estimate showed that steroid use

Table 1 Description of the included studies

First author	Country	Year	Subject number	Mean/median age (years)	Male patients (%)	BCG rate (%)	TST cut-off (mm)	Patients on IST (%)	IGRA method	ID rate of IGRA (%)
Andrisani	Italy	2013	92	39.6	50	1.1	5/10	76	QFT	1.1
Arenas Miras	Spain	2014	92	42.7	7.6	2.2	5/10	64.1	T-SPOT	4.3
Arias-Guillén	Spain	2014	205	42.3	50.2	89.8	5	83	QFT T-SPOT	2.4*
Bartalesi	Italy	2009	398	54.0	34.9	4.1	5/10	78	QFT	1.2
Casas	Spain	2011	214	48.5	49.7	23.6	5	76.2	QFT	5.6
Costantino	France	2013	563	51.0	43	78	5	83.3	T-SPOT	15.6
Hanta	Turkey	2012	90	40.9	48.9	92.2	5	47.8	QFT	N/A
Kwakernaak	Netherlands	2011	56	50.3	53.6	5	5	64.3	QFT	N/A
Laffitte	Switzerland	2009	50	48.0	70	90	5/10	68	T-SPOT	N/A
Mariette	France	2012	392	45.0	41.3	65.7	5	59.7	QFT T-SPOT	2.8*
Martyn-Simmons	UK	2013	70	47.0	58.6	64.3	5/15	43	QFT T-SPOT	4.3*
Matulis	UK	2008	142	47.9	50	83	5	89	QFT	5.6
Papay	Austria	2010	208	36.6	48.6	100	5/10	71.6	QFT	7.7
Schoepfer	Switzerland	2008	168	41.0	49.4	70.2	5/15	81	QFT	N/A
Scrivo	Italy	2012	119	47.0	31.1	5.9	5	80.7	QFT	17.6
Wong	Hong Kong	2014	265	43.1	59.7	73.1	5	46.6	QFT	1.5
Yilmaz	Turkey	2012	78	38.0	9	97.4	5	50	QFT	2.6

*Study that assessed both QFT and T-SPOT. The lower indeterminate rate is presented here.
BCG, Bacillus Calmette–Guérin; ID rate, indeterminate rate; IGRA, interferon γ release assay; IST, immunosuppressive therapy; N/A, not available; QFT, QuantiFERON-TB Gold In-Tube test; TST, tuberculin skin test.

Table 2 Details of immunosuppressive therapy in the included studies

First author	Drug	Percentage	Dose	Remarks
Andrisani	Azathioprine	41.3	2.5 mg/kg/day >3 months	
	Methotrexate	2.1	10–15 mg/week >3 months	
	Steroid	32.6	Prednisolone >20 mg/day for >2 weeks	
Arenas Miras	Prednisolone	19.5	>7.5 mg	
	Mycophenolate	N/A	243.1 mg (mean)	
	Methotrexate	N/A	1.22 mg (mean)	
Arias-Guillén	Immunosuppressant	31.2	Used within last 3 months	Azathioprine, 6-mercaptopurine, methotrexate
	Steroid	13.2	Used within last 2 weeks	
	Anti-TNF	15.6	Used within last 12 weeks	Infliximab or adalimumab
Bartalesi	DMARDs	61.6	N/A	Methotrexate, azathioprine, cyclosporine, leflunomide, cyclophosphamide, hydroxychloroquine
	Steroid	36.6	>4 weeks	
	Anti-TNF	24.2	N/A	Infliximab, etanercept, adalimumab
Casas	Methotrexate	42.5	>4 weeks	
	Leflunomide	16.8	>4 weeks	
	Cyclosporin A	10.3	>4 weeks	
	Others	6.1	>4 weeks	Azathioprine, efalizumab
	Steroid	42.5	>4 weeks	
Costantino	DMARDs	49.2	N/A	Methotrexate, leflunomide, others
	Steroid	45.1	10 mg/day	
	Anti-TNF	16.7	N/A	
Hanta	Immunosuppressant	N/A	N/A	
Kwakernaak	Methotrexate	48.2	19 mg (mean)	
	Leflunomide	5.4	20 mg (mean)	
	Azathioprine	1.8	150 mg (mean)	
	Steroid	5	6 mg (mean)	These 5% of patients received only steroid
Laffitte	Immunosuppressant	68	N/A	Methotrexate, cyclosporine, efalizumab
Mariette	Immunosuppressant	50.5	N/A	
	Steroid	59.7	N/A	
Martyn-Simmons	Cyclosporin	17.1	N/A	
	Methotrexate	15.7	N/A	
	Fumaric acid ester	2.9	N/A	
	Prednisolone	2.9	N/A	
	Combinational	2.9	N/A	
	Anti-TNF	5.7	N/A	
Matulis	DMARDs	70.4	N/A	Methotrexate, azathioprine, cyclosporine, leflunomide, cyclophosphamide, hydroxychloroquine, sulfasalazine, mycophenolate mofetil, sirolimus
	Steroid	40.1	N/A	
	Anti-TNF	59.2	N/A	Infliximab, etanercept, adalimumab
Papay	Immunosuppressant	47.1	Any dose >3 months	Thiopurines, methotrexate
	Steroid		Any dose >2 weeks	
	Anti-TNF		Any dose within last 12 weeks	
Schoepfer	Immunosuppressant	68.4	58% azathioprine >2 mg/kg, 18% 6-mercaptopurine >1 mg/kg, or 24% methotrexate >15 mg/week	
	Steroid	25.0	69% low dose (<20 mg daily) 29% moderate dose (20–40 mg daily) 2% high dose (40 mg daily)	
	Infliximab	14.9	N/A	
Scrivo	DMARDs	70.6	Methotrexate (range 0–25 mg), leflunomide (range 0–20 mg), cyclosporin (range 0–250 mg), sulfasalazine (range 0–3000 mg), azathioprine (range 0–100 mg), hydroxychloroquine (range 0–400 mg)	
	Steroid	64.7	Prednisone equivalent range 0–50 mg	
Wong	Azathioprine	35.1	Any dose	
	6-mercaptopurine	4.9	Any dose	
	Methotrexate	4.5	Any dose	
	Steroid	4.1	Prednisone >15 mg/day for >1 month	
Yilmaz	Hydroxychloroquine	57.5	N/A	
	Azathioprine	28.2	N/A	
	Mycophenolate mofetil	7.7	N/A	
	Cyclophosphamide	6.4	N/A	
	Methotrexate	2.6	N/A	
	Rituximab	1.3	N/A	
	Methylprednisolone	60.3	Methylprednisolone >10 mg/day	

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

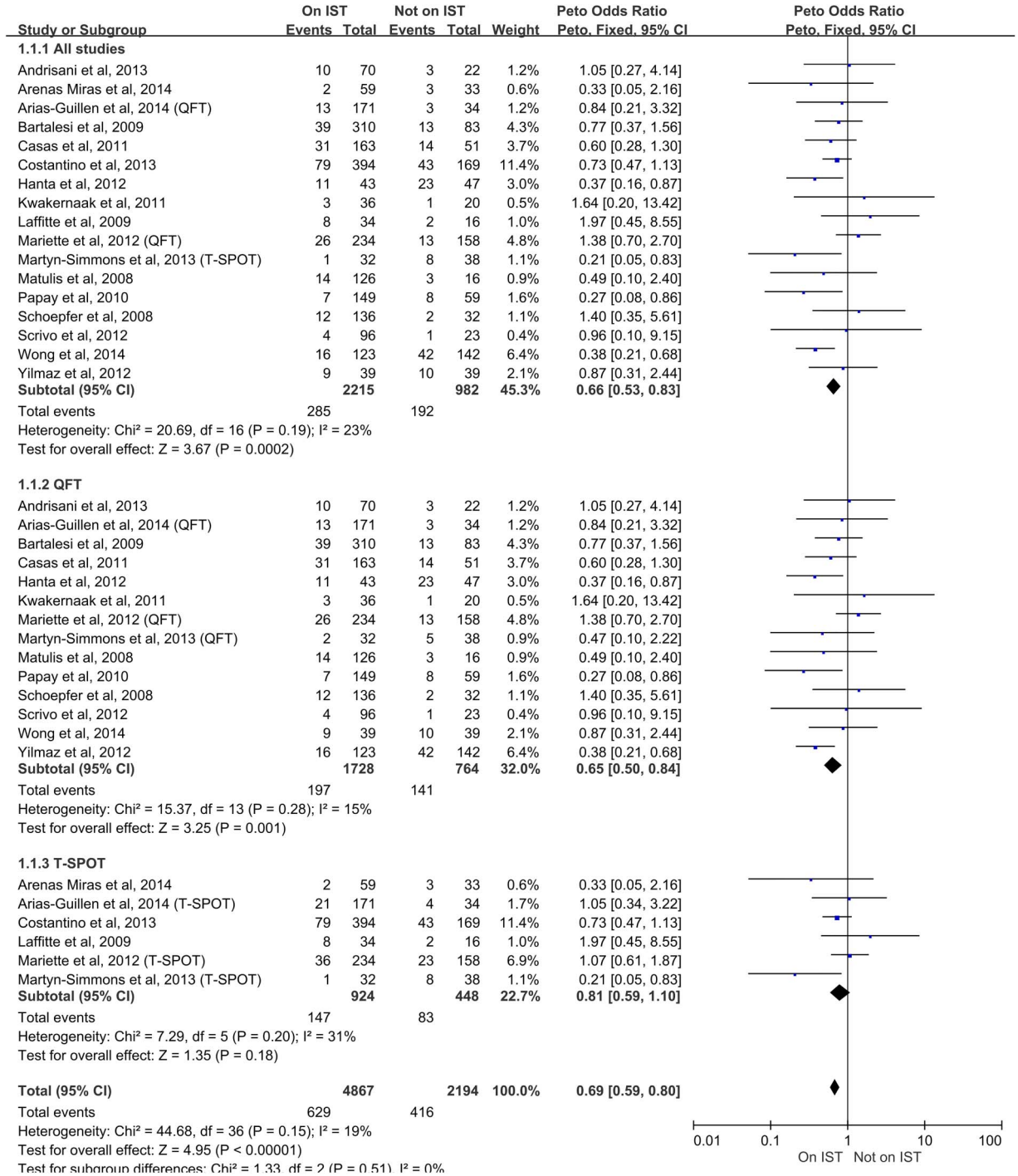

Figure 2 Forest plots of studies comparing positive interferon γ release assay (IGRA) results in patients with or without immunosuppressive therapy. The overall analysis (IGRA, top panel) and subgroup analyses (QuantiFERON-TB Gold In-Tube test (QFT), middle panel and T-SPOT, bottom panel) are shown.

was significantly associated with a lower rate of positive IGRA (OR 0.75, 95% CI 0.56 to 0.99) (figure 3). Furthermore, data from ten studies on oral immunosuppressants[12–14 16 18 20 22 23 25 26] and five studies on anti-TNF agents[13 14 22 23 26] showed a lower proportion of positive IGRA results, with an OR of 0.68 (95% CI 0.52 to 0.90) for oral immunosuppressants and an OR of 0.50 (95% CI 0.29 to 0.88) for anti-TNF therapy (figure 3).

Effect of immunosuppressive therapy on IGRA in different patient groups

We analysed the effect of immunosuppressive therapy on IGRA in two major groups of diseases: IBD and rheumatological diseases. The IGRA results were retrieved from five studies on IBD[11 12 23 24 26] and ten studies on rheumatological diseases.[12 14 16–19 21 22 25 27] We observed a consistent negative impact of immunosuppressive therapy on the IGRA results in

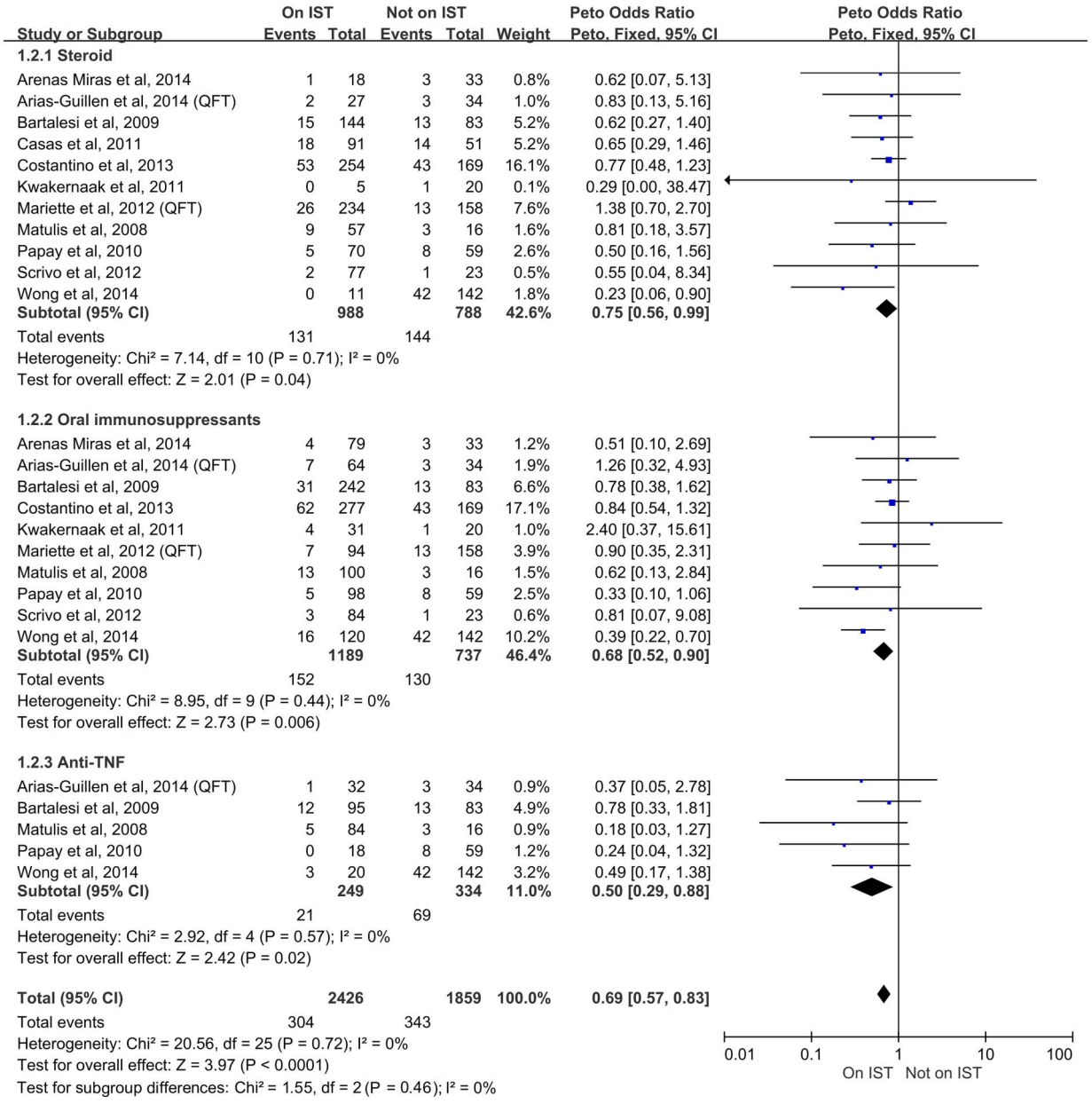

Figure 3 Forest plots of studies comparing positive interferon γ release assay (IGRA) results in patients with or without different kinds of immunosuppressive therapy. The subgroup analyses with steroids (top panel), oral immunosuppressants (middle panel) and anti-tumour necrosis factor (TNF) (bottom panel) are shown.

IBD (OR 0.50, 95% CI 0.32 to 0.77) and rheumatological disease (OR 0.67, 95% CI 0.50 to 0.90) groups (figure 4).

Effect of immunosuppressive therapy on TST in patients with autoimmune disease

We analysed the effect of immunosuppressive therapy on TST results in patients with autoimmune diseases. Most of the 16 studies showed lower rates of positive TST in patients taking immunosuppressive therapy, with significant associations observed in seven of the studies.[11–25 27] The rate of TST positive results were 23.2% and 39.5% among individuals with or without immunosuppressive therapy respectively. The pooled estimate showed that immunosuppressive therapy was significantly associated with a lower rate of positive TST (OR 0.51, 95% CI 0.42 to 0.61) with no significant heterogeneity between studies (see online supplementary figure S3).

DISCUSSION

Screening and treating LTBI is an important strategy to control the global spread of TB. There remains a knowledge gap in our understanding of the effect of immunosuppressive therapy on the performance of IGRA for LTBI screening.[28 29] To our knowledge, this is the first meta-analysis to assess the effect of immunosuppressive therapy on IGRA in patients with autoimmune disease.

In our meta-analysis, we identified a marked negative impact of immunosuppressive therapy on the IGRA results. In the studies, 71.5% of enrolled subjects were taking immunosuppressive therapy. This represents a common clinical practice nowadays. We found that the results of IGRA were strongly influenced by immunosuppressive therapy comprising steroids, oral immunosuppressants and biological therapy. This effect appeared to be more important in the QFT than the T-SPOT test with a lower Peto OR, although there were fewer studies

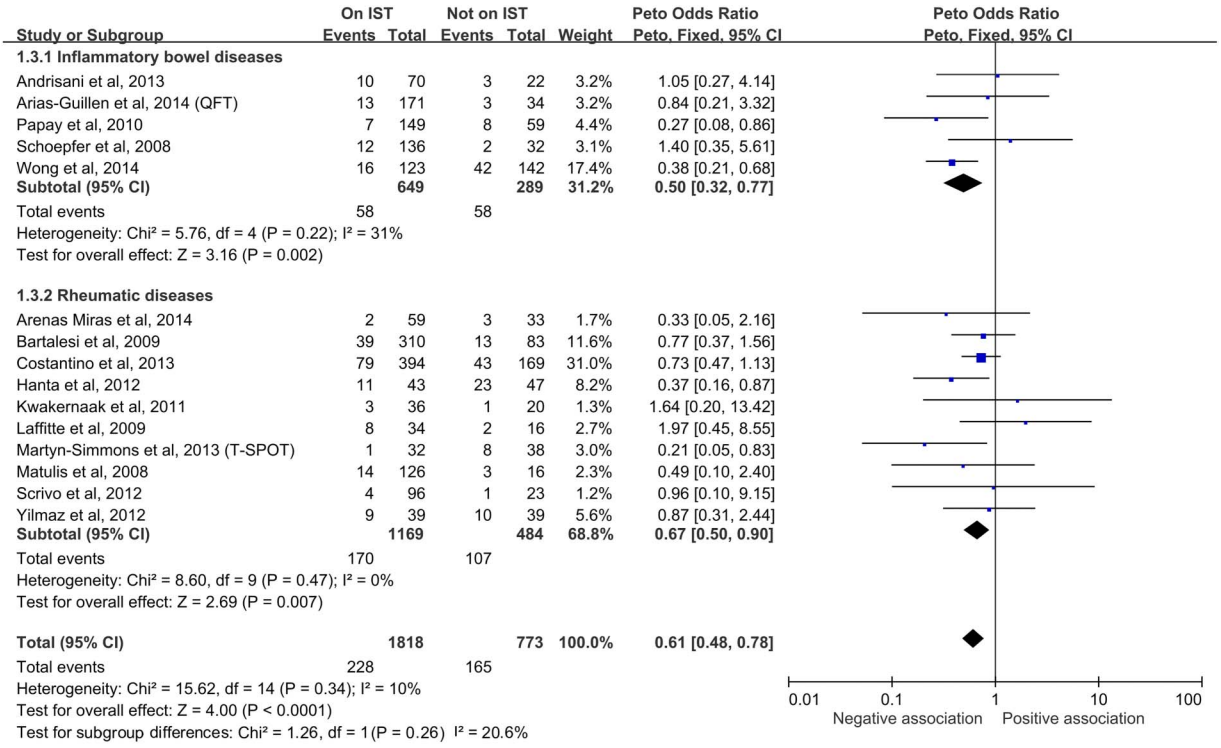

Figure 4 Forest plots of studies comparing positive interferon γ release assay (IGRA) results in patients with different groups of disease. The subgroup analyses in patients with inflammatory bowel diseases (top panel) and rheumatic diseases (bottom panel) are shown.

that assessed T-SPOT resulting in smaller statistical power. Although both methods measure interferon γ responses to TB-specific antigens (ESAT-6 and CFP-10) over a 16–24 h incubation period, some technical steps are different between the assays. Some studies reported a higher sensitivity with less indeterminate results with the T-SPOT test than the QFT method,[30 31] and it has been demonstrated that the use of a standardised number of washed peripheral blood mononuclear cells in the T-SPOT assay may contribute to its greater sensitivity. However, the measurement of interferon γ in the supernatant of whole blood in the QFT method would adversely affect results in patients with low T-cell counts, which may occur with immunosuppressive therapy.[32] Our study suggests a possible advantage of the T-SPOT method over the QFT method,[2] though more studies are required to make a fair comparison between the two tests.

The significant negative impact of anti-TNF therapy on the IGRA result is noteworthy. In clinical practice, it is recommended that patients be screened for LTBI before initiating anti-TNF therapy.[5] This study provides support for this recommendation, as potent immunosuppressive therapy can undermine the IGRA result significantly (Peto OR=0.50). This is likely due to the suppression of interferon γ and stimulation of immune cell apoptosis.[33 34] There is a concern that IGRA may not be sensitive in patients already on anti-TNF.

Furthermore, this negative impact on the IGRA results was also seen with other immunosuppressive agents, including steroids or oral immunosuppressants. This is consistent with some previous studies showing the negative impact of immunosuppressive therapy.[35–37] Moreover, two other papers suggested that about 7.5–10 mg/day of steroids may have enough suppressive effect on T-cell responsiveness which could negatively affect performance of immunodiagnostic tests for LTBI.[38 39] Although the results from some studies are conflicting,[13 18 20] our data favour a negative impact on the IGRA results from steroids and oral immunosuppressants with no significant heterogeneity between studies. This is in contrast to several individual studies claiming an absence of effect of IGRA,[12–14 21 22] despite each of them having insufficient power to detect such an effect. Assuming a true effect size with an OR of 0.75 for steroids as estimated in this meta-analysis, up to 1617 samples are required to achieve a power of 80% at an α error rate of 5%. It is likely that even the largest study,[16] with a total sample size less than one-third of this estimation, would be inadequate to conclude the steroid effect on IGRA. Combining data from 11 studies with 1776 subjects, our meta-analysis offered a greater power than any of the individual studies to detect a significant effect. Nevertheless, it is noteworthy that the studies used medications at different doses with different routes of administration, and the heterogeneity may not have been captured in the statistical analyses.

Another interesting finding is that although immunosuppressive therapy strongly effects the performance of IGRA, it appears to have no significant correlation with the rates of indeterminate results. This may be explained by patient-related or test-related factors. Low peripheral blood lymphocyte count, low serum albumin and treatment with immunosuppressive therapy were reported as predictors of indeterminate results.[40] Furthermore, the overall rate of indeterminate results is relatively low, thus affecting the power to detect a significant difference. In this analysis, only two of the nine studies reported an indeterminate rate of above 10%,[16 25] and the average indeterminate rate of the remaining seven studies was around 5%. Despite a lower IGRA positive rate, we did not observe a higher indeterminate rate among patients on immunosuppressive therapy, suggesting that the drugs may have undermined the test to produce more negative but not indeterminate results.

Our study has several limitations. First, despite subgroup analyses of different classes of immunosuppressive therapy, the

effect of individual therapeutic agents could not be ascertained due to the insufficient raw data. For example, only three studies have provided the raw data for thiopurine or methotrexate[18 23 26]; and only four studies on steroids mentioned the dosage used (5–10 mg per day respectively[12 15 18 26]). Further stratified analyses on these individual immunosuppressants cannot be calculated from the original publications. Second, age may also affect the likelihood of positive or indeterminate IGRA results. However, as the raw data for each individual study with and without immunosuppressive therapy were not available, we were unable to assess the effect of age as a potential confounder in a meta-regression. Nevertheless, given the relatively young age of subjects in the individual studies (range 36.6–54.0, table 1), this effect is likely to be limited. Third, as we compared individuals with autoimmune diseases, there could be other possible confounding factors associated with the immunosuppressive therapy. These factors may include the different formulations of medications, disease activity and associated comorbidities. These effects may not be captured in the statistical analyses due to the heterogeneity of study design and limited available data.

In conclusion, this meta-analysis showed a pronounced negative impact of immunosuppressive therapy on the IGRA results. Our findings suggest that IGRA may not be reliable in the diagnosis of LTBI of patients on immunosuppressive therapy, including anti-TNF, steroids and oral immunosuppressants. Patients should preferably be screened for LTBI before initiation of immunosuppressive therapy. These results would be useful to physicians in pulmonology, infectious disease, rheumatology, gastroenterology and dermatology specialties in understanding how reliable IGRA is when tested in patients already on some forms of immunosuppressants.

Author affiliations
[1]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, Hong Kong
[2]State Key Laboratory of Digestive Diseases, Institute of Digestive Disease, LKS Institute of Health Science, The Chinese University of Hong Kong, Shatin, Hong Kong
[3]Division of Gastroenterology and Hepatology, Ren-Ji Hospital, Shanghai Jiao-Tong University School of Medicine, Shanghai Institute of Digestive Disease, Shanghai 200240, China
[4]Faculty of Medicine, School of Public Health and Primary Care, Institute of Digestive Disease, The Chinese University of Hong Kong, Shatin, Hong Kong
[5]Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Shatin, Hong Kong

Contributors SHW, QG and SCN take responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version of the manuscript. Study concept and design: SHW, QG and SCN. Acquisition, analysis, or interpretation of data: SHW, QG, KKFT, WKKW and SCN. Drafting of the manuscript: SHW, QG, L-sT, NL, JJYS and SCN. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: SHW, QG, KKFT and WKKW. Study supervision: L-sT, NL, FKLC, JCYW, JJYS and SCN.

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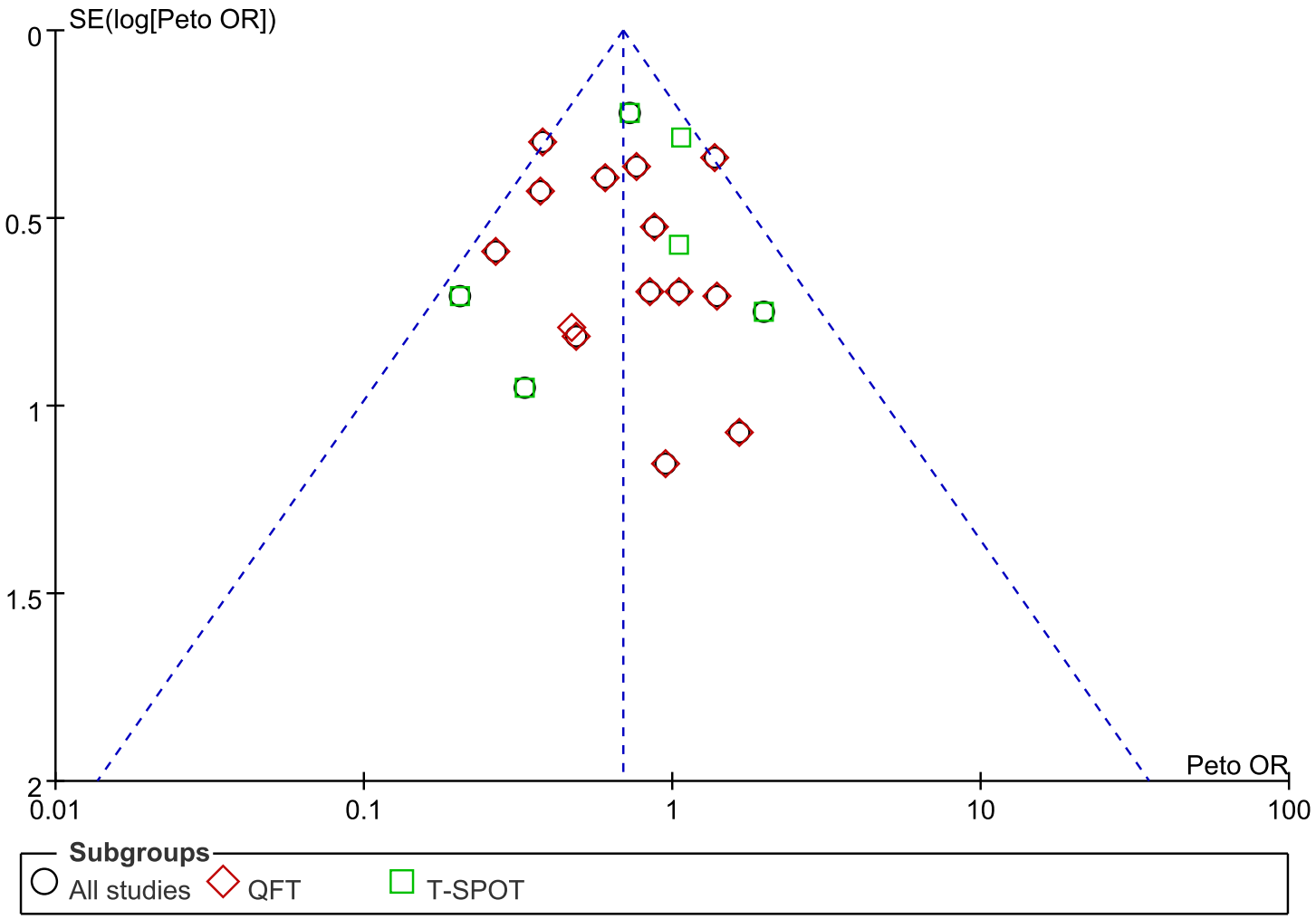
Supplementary Figures

***Supplementary Figure 1*.** Funnel plot of studies included in the primary outcome analysis.

***Supplementary Figure 2*.** Forest plots of studies comparing indeterminate IGRA results in patients with or without immunosuppressive therapy.

Supplementary Figure 3. Forest plots of studies comparing positive TST results in patients with or without immunosuppressive therapy.


SE(log[Peto OR])
0
0.5
1
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0.01
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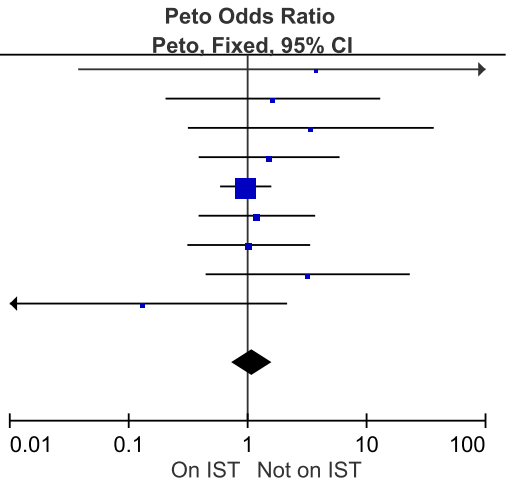
Peto OR
Subgroups
All studies
QFT
T-SPOT

Study or Subgroup	On IST Events	On IST Total	Not on IST Events	Not on IST Total	Weight	Peto Odds Ratio Peto, Fixed, 95% CI
Andrisani et al, 2013	1	70	0	22	0.7%	3.72 [0.04, 368.40]
Arenas Miras et al, 2014	3	59	1	33	3.3%	1.63 [0.20, 13.02]
Arias-Guillen et al, 2014 (QFT)	5	171	0	34	2.5%	3.40 [0.31, 36.70]
Casas et al, 2011	10	163	2	51	7.8%	1.52 [0.39, 5.93]
Costantino et al, 2013	61	394	27	169	58.6%	0.96 [0.59, 1.58]
Papay et al, 2010	12	149	4	59	11.3%	1.20 [0.39, 3.70]
Scrivo et al, 2012	17	96	4	23	10.2%	1.02 [0.31, 3.35]
Wong et al, 2014	3	123	1	142	3.7%	3.20 [0.44, 23.08]
Yilmaz et al, 2012	0	39	2	39	1.9%	0.13 [0.01, 2.15]
Total (95% CI)		**1264**		**572**	**100.0%**	**1.10 [0.75, 1.61]**
Total events	112		41			

Heterogeneity: Chi² = 5.14, df = 8 (P = 0.74); I² = 0%

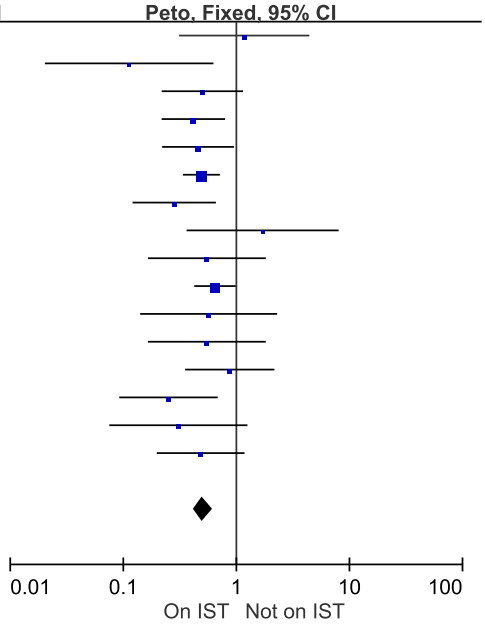
Test for overall effect: Z = 0.49 (P = 0.63)



Study or Subgroup	On IST Events	Total	Not on IST Events	Total	Weight	Peto Odds Ratio Peto, Fixed, 95% CI
Andrisani et al, 2013	11	70	3	22	2.1%	1.17 [0.31, 4.42]
Arenas Miras et al, 2014	1	59	5	33	1.3%	0.11 [0.02, 0.63]
Arias-Guillen et al, 2014 (QFT)	42	171	13	34	5.4%	0.50 [0.22, 1.15]
Bartalesi et al, 2009	52	319	22	74	8.9%	0.42 [0.22, 0.79]
Casas et al, 2011	34	163	18	51	6.9%	0.46 [0.22, 0.95]
Costantino et al, 2013	118	394	78	169	26.0%	0.49 [0.34, 0.72]
Hanta et al, 2012	20	43	36	47	5.2%	0.28 [0.12, 0.66]
Kwakernaak et al, 2011	6	36	2	20	1.6%	1.71 [0.36, 8.03]
Laffitte et al, 2009	12	34	8	16	2.6%	0.55 [0.17, 1.82]
Mariette et al, 2012 (QFT)	73	234	65	158	20.9%	0.65 [0.42, 0.99]
Martyn-Simmons et al, 2013 (T-SPOT)	3	32	6	38	1.9%	0.57 [0.14, 2.29]
Matulis et al, 2008	12	34	8	16	2.6%	0.55 [0.17, 1.82]
Papay et al, 2010	18	149	8	59	4.5%	0.87 [0.35, 2.17]
Schoepfer et al, 2008	19	136	11	32	3.7%	0.25 [0.09, 0.68]
Scrivo et al, 2012	9	96	5	23	1.9%	0.31 [0.08, 1.25]
Yilmaz et al, 2012	15	37	23	39	4.7%	0.48 [0.20, 1.18]
Total (95% CI)		**2007**		**831**	**100.0%**	**0.51 [0.42, 0.61]**
Total events	445		311			

Heterogeneity: Chi² = 14.27, df = 15 (P = 0.51); I² = 0%

Test for overall effect: Z = 6.93 (P < 0.00001)



Supplementary Table 1. Modified QUADAS quality assessment of the included studies.

First author	Item 1	Item2	Item4	Item 8	Item12	Item13	Item14
Andrisani	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Arenas Miras	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Arias-Guillén	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Bartalesi	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Casas	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Costantino	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hanta	Yes	Unclear	Unclear	Yes	Yes	No	Yes
Kwakernaak	Yes	Unclear	Yes	Yes	Yes	No	Yes
Laffitte	Yes	Unclear	Unclear	Yes	Yes	No	Yes
Mariette	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Martyn-Simmons	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes
Matulis	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Papay	Yes	Yes	Unclear	Yes	Yes	Yes	Yes
Schoepfer	Yes	Yes	Yes	Yes	Yes	No	Yes
Scrivo	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes
Wong	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes
Yilmaz	Yes	Yes	Unclear	Yes	Yes	Yes	Yes

Item 1: Was the spectrum of patients representative of the patients who will receive the test in practice?
Item 2: Were selection criteria clearly described?
Item 4: Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?
Item 8: Was the execution of the index test described in sufficient detail to permit replication of the test?
Item 12: Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?
Item 13: Were uninterpretable/ intermediate test results reported?
Item 14: Were withdrawals from the study explained?