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QuantiFERON TB Gold Plus for the diagnosis of tuberculosis: a systematic review and meta-analysis

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SUMMARY

Estimated 2017 tuberculosis (TB) incidence is 10 million and mainly depends on the reservoir of individuals with latent TB infection (LTBI). Quantiferon®-TB Gold in-Tube (QFT-GIT) is one of the tests used for LTBI detection. Since 2015 a new version, Quantiferon®-TB Gold Plus (QFT-Plus) is available.

Objectives: To perform a systematic review and meta-analysis to assess the diagnostic accuracy for TB of QFT-Plus compared to QFT-GIT.

Methods: PubMed and Scopus were used to detect records related to predefined strings from 2015 to 2018. Full text articles dealing with the sensitivity and/or specificity of the QFT-Plus vs. QFT-GIT for active-TB and LTBI detection were analyzed. Scientific quality and risk of bias were assessed using QADAS-2.

Results: We selected 15 articles. Studies were mainly observational and cross-sectional, performed in 8 countries. Sample size differed in the TB group (27 to 164) compared to LTBI group (29 to 1031). Pooled sensitivity of QFT-Plus for active-TB was 0.94 (0.91 and 0.95 for TB1 and TB2, respectively), whereas pooled specificity for healthy status was 0.96. Pooled sensitivity and specificity for LTBI was 0.91 and 0.95, respectively.

Conclusions: We show that QFT-Plus is more sensitive compared to QFT-GIT for detecting M. tuberculosis infection, mainly due to TB2 responses.

Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is still an important global health problem with 10 million cases and 1.6 million deaths worldwide estimated in 2017 by the World Health Organization (WHO).[1–5] Almost one-fourth of the world's population is latently infected with Mtb with a risk of progression to active disease of about 3–15% during their lifetimes.[2,6,7] Being latent TB infection (LTBI) an important reservoir for TB disease progression, an effectively elimination of TB epidemic is feasible only diagnosing and treating LTBI.[8–13]

Tuberculin skin test (TST) and T-cell interferon-γ release assays (IGRAs) are the routine tests used for LTBI diagnosis[9]; to note that no gold standard tests for LTBI diagnosis exist and therefore active TB is used as a surrogate to measure the sensitivity of these assays. The TST is based on an immune reaction to the intradermal injection of purified protein derivate (PPD) of tuberculin, a mixture of antigens that, however, is shared by Mtb and bacille Calmette–Guérin (BCG) as well as other mycobacteria, affecting the specificity of the test.[14,57–59]

To overcome this limitation, IGRAs have been developed.[60–62] Two IGRAs, Quantiferon®-TB Gold in Tube (QFT-GIT) and T-Spot®.TB (TSPOT), are available.[13,15–18] IGRAs measure (by enzyme-linked immune-assay and by enzyme-linked immunospot, respectively) the IFN-γ production to Mtb specific peptides ESAT-6, CFP-10 and TB7.7 (one only in QFT-GIT) located in the region of difference (RD-1 and RD-11) of Mtb. However, also the IGRAs may show cross reactivity with mycobacteria containing antigens within the RD1 region[19] and have higher costs compared to TST.[17,20] Furthermore, since both TST and IGRAs imply an immune reaction, these tests have a suboptimal diagnostic accuracy in immunocompromised patients as well as in children who have an immature immune system.[11,21,22] Finally, TST and IGRAs do not differentiate between active TB and LTBI, between active TB and cured TB,[23–25] and importantly, between recent and remote latent infections.[7,26–28] Such differentiation is greatly important, since the risk of progress to active disease is highest in the first two years after Mtb exposure.[13]

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Table 1
Summary of the selected studies on QFT-Plus.

	First author	Title	Publication year	Type of study	Country	Mono-/multi-center study	Study period
(1)	Agarwal[41]	Performance and variability of QuantiFERON Gold Plus assay associated with phlebotomy Type	2018	Observational	USA	Monocentre	2017
(2)	Barcellini[54]	First evaluation of QuantiFERON-TB Gold Plus performance in contact screening	2016	Cross-sectional	Italy	Monocentre	Nov 2014–Jun 2015
(3)	Chien[43]	Quantiferon-TB Gold Plus is a More Sensitive Screening Tool than Quantiferon-TB Gold In-Tube for Latent Tuberculosis Infection among Older Adults in Long-Term Care Facility	2018	Observational	Taiwan	Multicentre	Jun–ul 2017
(4)	Gallegos Morales[50]	Prevalence of latent tuberculosis infection among foreign students in Lubeck, Germany tested with QuantiFERON-TB Gold In-Tube and QuantiFERON-TB Gold Plus.	2017	Cross-sectional	Germany	Monocentre	Feb-Mar 2016
(5)	Hoffmann[51]	Equal sensitivity of the new generation QuantiFERON-TB Gold plus in direct comparison with the previous test version QuantiFERON-TB Gold IT	2016	Observational	Germany	Monocentre	Jul 2015–Jan 2016
(6)	Horne[44]	Multicentre study of QuantiFERON-TB Gold Plus in patients with active tuberculosis	2018	Cross-sectional	USA/Japan	Multicentre	Oct 2013–Sep 2016
(7)	Moon[47]	Evaluation of Quantiferon-TB Gold-Plus in Healthcare Workers a Low-Incidence Setting	2017	Cross-sectional	USA	Monocentre	Aug 2015–Nov 2015
(8)	Petruccioli[48]	Analytical evaluation of QuantiFERON-Plus and QuantiFERON-Gold In-tube assays in subjects with or without tuberculosis	2017	Observational	Italy	Monocentre	-
(9)	Petruccioli[53]	First characterization of the CD4 and CD8 T-cell responses to QuantiFERON-TB Plus	2016	Observational	Italy	Monocentre	-
(10)	Pieterman[46]	A multicentre verification study of the QuantiFERON®-TB Gold Plus assay	2018	Verification	The Netherlands/ Belgium	Multicentre	May 2015–Dec 2016
(11)	Ryu[45]	Comparative Evaluation of QuantiFERON-TB Gold In-Tube and 2 QuantiFERON-TB Gold Plus in the Diagnosis of Latent Tuberculosis Infection in Immunocompromised Patients	2018	Prospective cohort	Korea	Monocentre	Feb-Aug 2017
(12)	Siegel[40]	Specificity of QuantiFERON-TB Plus, a New-Generation Interferon Gamma Release Assay	2018	Observatinal	USA	Monocentre	-
(13)	Takasaki[49]	Sensitivity and specificity of QuantiFERON-TB Gold Plus compared with QuantiFERON-TB Gold In-Tube and T-SPOT.TB on active tuberculosis in Japan	2017	Case-control	Japan	Monocentre	Feb 2014–Dec 2014
(14)	Theel[42]	Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube Interferon-γ Release Assays in Patients at Risk for Tuberculosis and in Healthcare Workers.	2018	Cohort	USA	Monocentre	Jun 2016–Apr 2017
(15)	Yi[52]	Evaluation of QuantiFERON-TB Gold Plus for Detection of Mycobacterium tuberculosis infection in Japan	2016	Observational	Japan	Multicentre	Jan 2014–Mar 2015

IGRAs are based on the assumption that CD4 T-cells play a critical role in the immune response to Mtb.[29] However, recent evidences support the idea that also CD8 T-cells are an important component of host immunity to Mtb.[30,31] Indeed, CD8 T-lymphocytes are able to mount an immune response producing IFN-γ in vitro after stimulation with Mtb antigens.[32–34] Moreover, RD1-specific CD8+ *T* cells are more frequently detected in active TB patients than in subjects with LTBI[30,31,35] and after recent infection compared to remote latent infection.[18]

A new version of QFT-GIT is available since 2015, the Quantiferon®-TB Gold Plus (QFT-Plus) which has been proposed as the 4th generation Quantiferon. QFT-Plus contains two TB-specific antigen tubes, called TB1 and TB2: the TB1 tube, contains long peptides derived from ESAT-6 and CFP-10 (TB-7.7, present in the previous QFT-GIT version has been removed), and it is designed to induce a specific CD4 T-cell response; TB2, besides the same long peptides of TB1, contains also shorter peptides able to stimulate CD8 T-cells.[36] This new test, overcoming the dependence on the CD4-mediated responses alone, could potentially have an increased sensitivity for the diagnosis of LTBI in HIV-infected subjects. Moreover, as CD8 T-cell responses seem to work at the early phase of Mtb infection and at a phase of reactivation from LTBI,[18,31,37,38] QFT-Plus test might be useful in differentiating recent and remote LTBI, helping in the decision to start LTBI treatment.

In the last 3 years, several studies were performed to assess the diagnostic accuracy of QFT-Plus over another routine immune diagnostic test (TST, QFT-GIT, T Spot TB). As reported above, active TB cases were used as a surrogate to evaluate the sensitivity of the test for LTBI diagnosis.

Aim of this study was to assess the diagnostic accuracy of QFT-Plus in TB and LTBI in comparison with QFT-GIT, carrying out a systematic review and meta-analysis.

Methods

Search strategy

A search of the scientific literature was carried out to select full-text articles describing findings of observational studies on the

Table 2
Criteria that were used to select the populations for inclusion in the studies.

Study	TB	LTBI	Population classified "NO TB" at enrolment
Agarwal[41]	-	Positive TST	HCWs (epidemiological criteria)
Barcellini[54]	-	Recent contacts that were TST-positive (≥5 mm)	Students
Chien[43]	-	-	Elderly residents in long-term care facilities
Gallegos Morales[50]	-	-	Migration background
Hoffmann[51]	TB-related clinical presentation; Positive microbiology.	-	HCWs
Horne[44]	Positive culture.	-	-
Moon[47]	Bacteriological confirmed active TB	-	Healthy donors (epidemiological criteria)
Petruccioli[48]	-	-	HCWs
Petruccioli[53]	TB-related clinical presentation; Positive microbiology; TB-related radiological signs.	Positive IGRA (QFT-GIT).	Healthy donors (QFT-IT negative)
Pieterman[46]	TB-related clinical presentation; Positive microbiology.	Positive IGRA (QFT-GIT).	Healthy donors (QFT-IT negative)
Ryu[45]	-	-	Subjects undergoing QFT-screening (contact investigation, differential diagnosis for LTBI, immuno-therapy, periodic check-up in HCWs)
Siegel[40]	-	-	SOT, HSCT, TNF-inhibitors treated subjects
Takasaki[49]	-	-	Healthy donors (epidemiological criteria); NTM patients
Theel[42]	TB-related clinical presentation; Positive microbiology; TB-related radiological signs.	-	Healthy donors (epidemiological criteria)
Yi[52]	Positive microbiology (culture; PCR).	-	HCWs (TST-negative: <10 mm)

Table 3
Demographic and epidemiological characteristic of the selected studies in which the gender information was available.

Study	TB patients		LTBI subjects		Population classified as "no TB" at the end of the study	
	Sample size, *n*	Male, *n* (%)	Sample size, *n*	Male, *n* (%)	Sample size, *n*	Male, *n* (%)
Agarwal[41]	-	-	299	89 (31.5)	-	-
Barcellini[54]	-	-	119	63 (52.9)	-	-
Chien[43]	-	-	229	117 (51.5)	-	-
Gallegos Morales[50]	-	-	134	62 (46.3)	-	-
Hoffmann[51]	57	-	-	-	77	-
Horne[44]	164	100 (61)				
Moon[47]	-	-	989	301 (30.4)	-	-
Petruccioli[48]	27	14 (51.9)	30	11 (36.7)	10	-
Petruccioli[53]	69	41 (59.4)	58	28 (48.3)	19	9 (47.4)
Pieterman[46]	-	-	1031	414 (40.0)	-	-
Ryu[45]	-	-	317	205 (64.7)	-	-
Siegel[40]	-	-	-	-	211	54 (25.6)
Takasaki[49]	99	65 (65.7)	-	-	106	-
Theel[42]	-	-	29	-	-	-
Yi[52]	162	129 (79.6)	-	-	212	105 (49.5)

diagnostic accuracy of QFT-Plus in individuals with suspected TB disease and LTBI in whom QFT-GIT was performed.

The databases PubMed and Scopus were used to detect records associated with articles published in peer-reviewed medical journals from its inception to 31st December 2018.

A list of keywords and their combination in strings was prepared following the research question of the study:

"QFT-Plus", "QFT-IT", "TB", "LTBI", and "Diagnosis".

The sensitivity of the search was improved with the assessment of the lists of references of the selected articles and manuscripts included in reviews, systematic reviews and meta-analyses on QFT.

Findings of national and international abstracts and of articles of grey literature were not kept into consideration, following the poor information on methods and results and the missing peer-review assessment, respectively.

Study selection

Articles describing studies on the sensitivity and/or specificity of the QFT-Plus in comparison with QFT-GIT in patients with TB disease and/or in individuals with LTBI, and/or in healthy subjects were considered suitable for the qualitative and quantitative analysis.

Exclusion criteria were the following: (a) articles on animal- or modelling-based studies; (b) editorials, correspondences, narrative and systematic reviews; (c) articles published in languages other than English; (d) case-reports or case-series with less than ten patients; (d) articles describing studies only on QFT or not aimed at assessing the diagnostic accuracy of QFT-Plus.

Titles and abstracts of the records were carefully and independently evaluated by two study Authors (L.S. and G.S.). Full texts were independently assessed by the same Authors and potential disagreement in the process of article selection and data extraction were resolved by consensus and by the support of the additional Authors (D.G.).

Data extraction

Aggregated data from selected manuscripts were retrieved with an ad hoc electronic form, which included section on demographics, epidemiology, study methodology, and diagnostic accuracy (i.e., sensitivity, specificity, positive and negative predictive values). In particular, the following variables were included in the above-mentioned form: study authors; study title; publication year; type of epidemiological study; country/ies and clinical centre/s where the study was carried out; study period when the study was

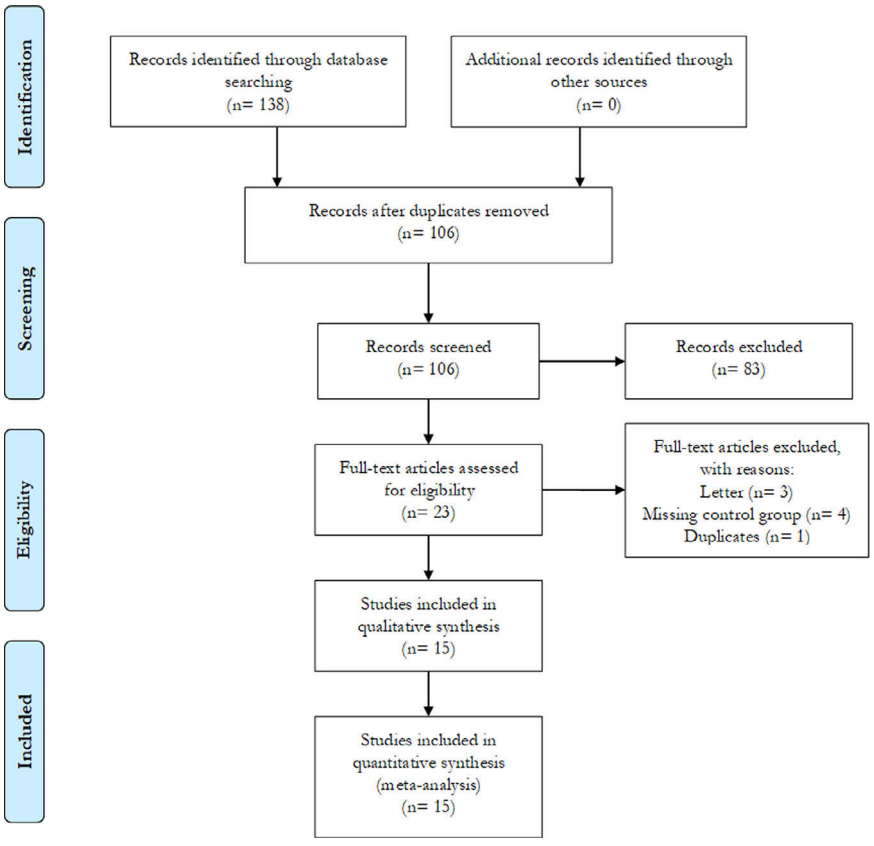
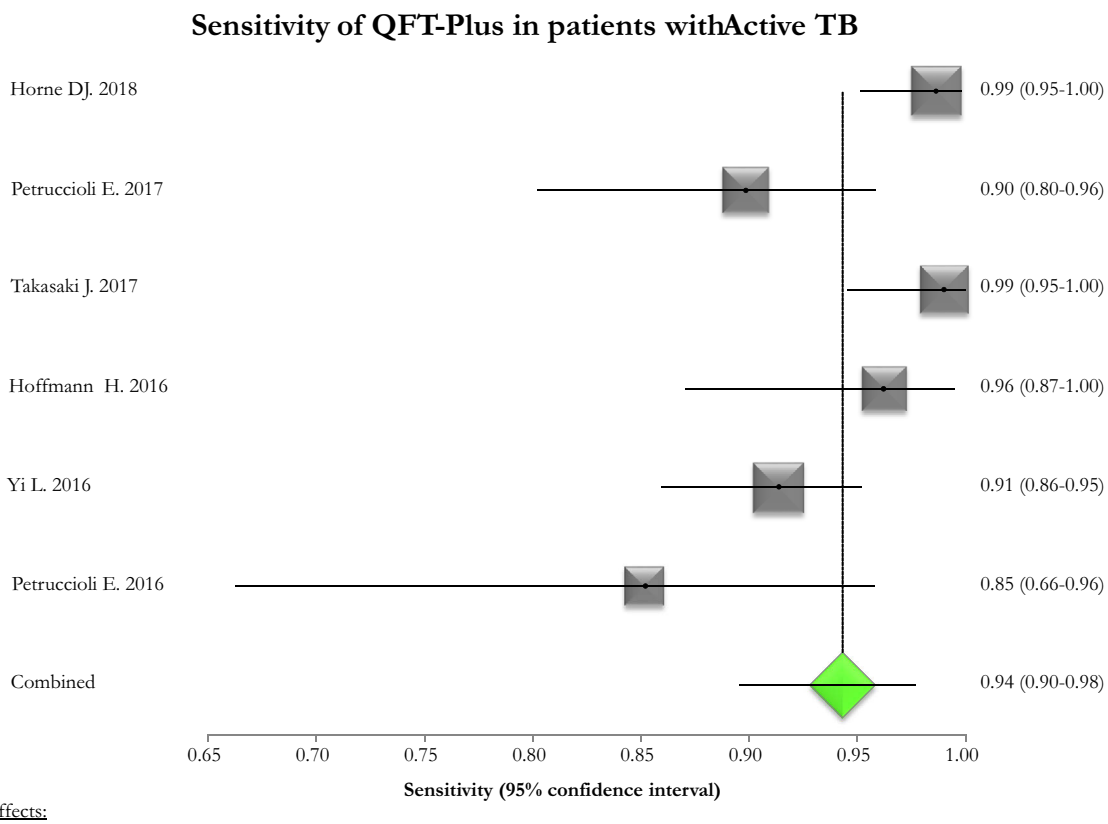


Fig. 1. PRISMA 2009 flow diagram.



Random effects:
Pooled proportion= 0.94 (95% CI = 0.90 to 0.98)
I^2= 75.8% (95% CI = 27.3% to 87.5%)

Fig. 2. Sensitivity of QFT-Plus in patients with active tuberculosis.

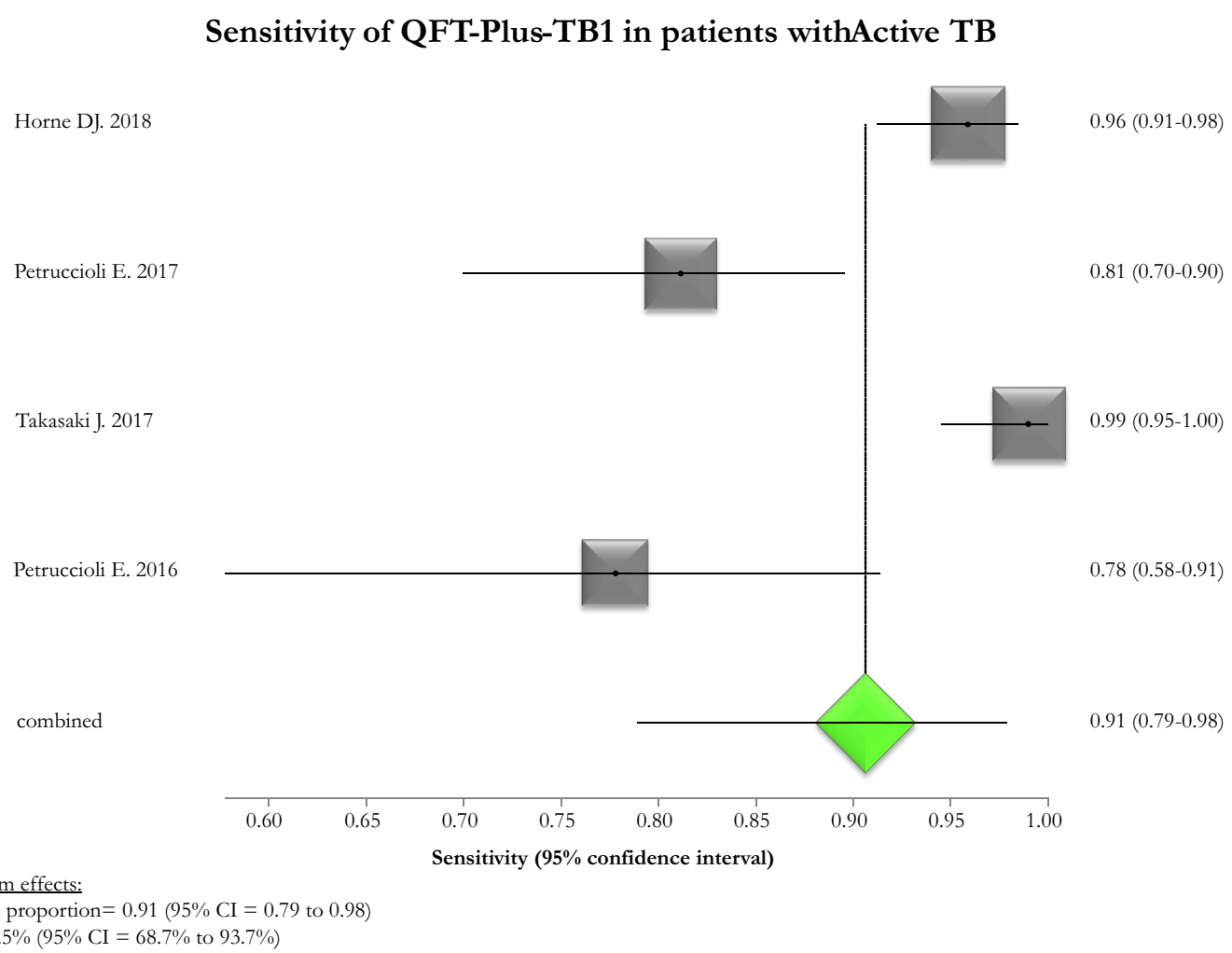


Fig. 3. Sensitivity of QFT-Plus-TB1 in patients with active tuberculosis.

performed; sample size; gender; diagnostic methods prescribed for LTBI and TB disease; frequency of true positives and negatives, and of false positives and negatives.

Data retrieved from the papers did not describe single patients or individuals: information were anonymized and aggregated. Then, no ethical approval was needed.

No relevant discrepancies were detected in the search and in the extraction phases, with an inter-rater agreement of 100%.

Study quality assessment

The present systematic review and meta-analysis was performed following the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (Fig. 1) (PRISMA).[39]

Scientific quality and risk of bias was assessed using the QADAS-2. It consists of four domains aimed at assessing the risk of bias: patients' selection, index test, reference standard, and flow and timing. The first three domains assess the applicability. The risk of bias was ranked as low, high, or unclear.

Statistical analysis

Qualitative and quantitative analyses were performed. Qualitative variables were summarized with absolute (relative) frequencies, whereas quantitative variables were described with means (standard deviations, DS) or medians (interquartile ranges, IQR).

Meta-analytic estimates were computed and described with pooled and heterogeneity indicators.

Forest plots were adopted to show between-study variability of point estimates and their 95% confidence intervals (CI), as well as the weight of the sample sizes. The inconsistency indicator (I^2) summarized the relationship between true variability and total variation. Low, medium, and high heterogeneity was represented by I^2 values of <25%, ≥25%-<50%, and ≥50%.Potential publication bias was investigated through bias assessment plots and the Egger weighted regression test method.

Fixed or random effects models were chosen based on the estimated between-study heterogeneity.

A two-tailed p-value less than 0.05 was deemed statistically significant.

All computations were performed with STATA version 15 (StataCorp, Lakeway Drive, College Station, Tx, USA) and StatsDirect version 3.1.12 (StatsDirect Ltd.) statistical softwares.

Results

Characteristics of the selected studies

The selected 15 articles were published between 2016 and 2018[40–54]; they describe studies carried out between 2013[43] and 2017.[41–43,45] (Table 1) The epidemiological design of these observational reports was clearly described only in 8 studies: 4 (27%)

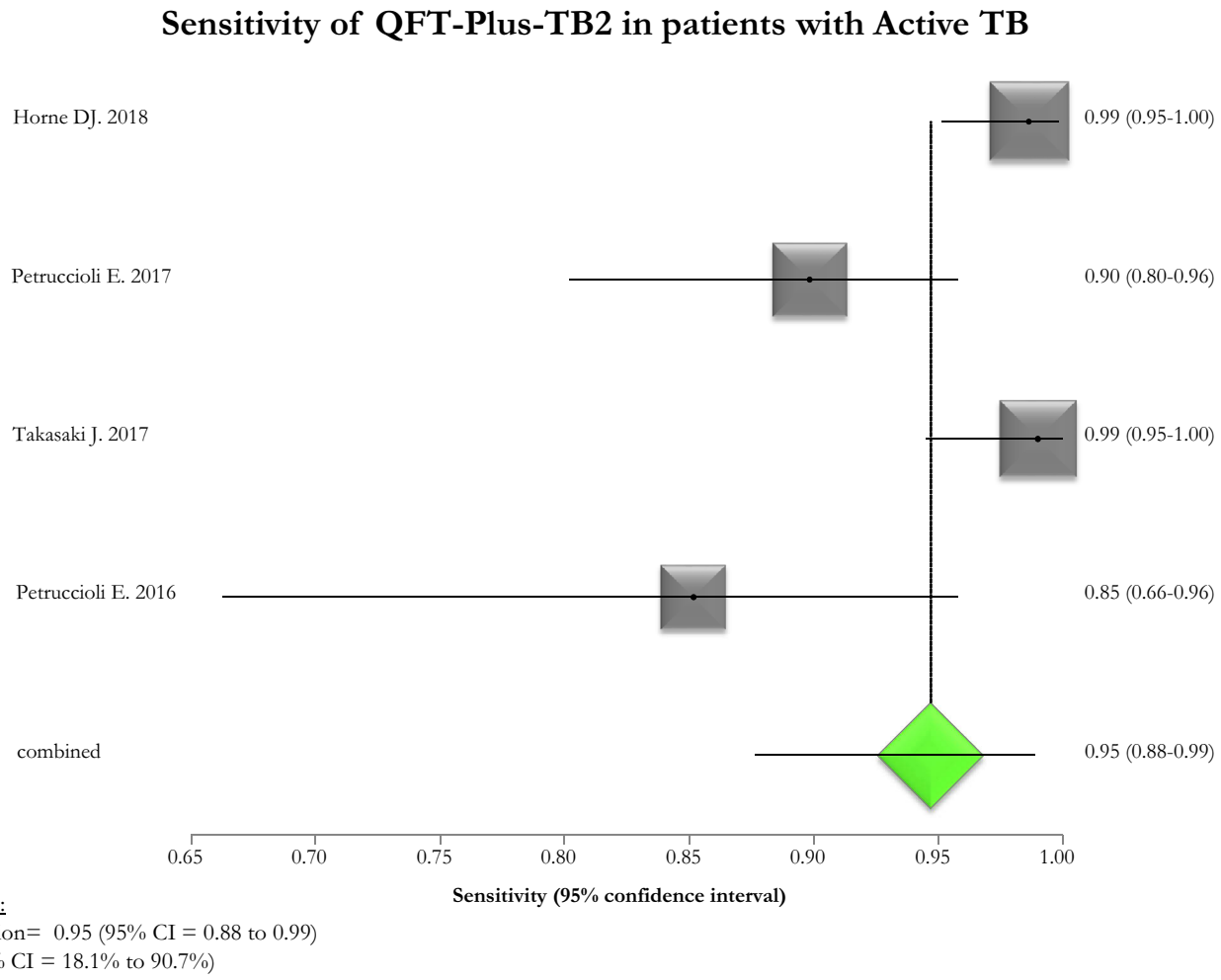


Fig. 4. Sensitivity of QFT-Plus-TB2 in patients with active tuberculosis.

were cross-sectional,[44,47,50,54] 2 (13%) were cohort studies,[42,45] 1 (7%) was a verification study,[46] and 1 (7%) a case-control study.[49] It was not reported the analytical design of the remaining 7 studies (47%).[40,41,43,48,51–53] The clinical centres where the studies were conducted were in 8 countries worldwide: 4 (50.0%) in Europe[46,48,50,51,53,54] [i.e., 3 (42.9%) in Italy,[48,53,54] 2 (28.6%) in Germany,[50,51] 1(14.3%) in the Netherlands,[46] 1 (14.3%) in Belgium[46]], 3 (37.5%) in Asia[43–45,52] [3 (60%) in Japan,[44,49,52] 1 (20%) in Taiwan,[43] 1 (20%) in Korea,[45] and 1 (12.5%) in North America[41,43,46] [5 (100%), in USA[40–42,44,47]]. The majority [11 (73.3%)] of the studies were carried out in single clinical centres[40–42,45,47–51–53] and only 4 were multi-centre.[43,44,46,52]

TB disease was diagnosed in selected studies as by clinical means,[48,49,51,53] and/or radiological[49–53] signs and/or positive microbiology tests (e.g., conventional and/or molecular assays)[42,44,48,49,51,53] (Table 2). LTBI was diagnosed based on positive TST[41,42,45,54] and/or IGRA[42,43,45,46,48,50,53] (i.e., QFT[42,43,45,46,48,50,53]).

Characteristics of the study samples

Sample size ranged depending on tested categories: it ranged from 27[53] to 164[44] patients in the TB group, from 29[42] to 1031[46] in the LTBI group, and from 10[53] to 212[52] in the healthy donors group (Table 3).

As reported inTable 3 in which we selected the papers in whom the gender was mentioned, TB patients were mainly male, as reported in 5/8 (62.5%) studies[44,48,49,52,53] (range: 51.9%[53]–79.6%[52]), whereas healthy donors were mainly female (as shown in 3/15 (20.0%) studies[40,48,52]; range: 50.5%[52]–74.4%[40]). The proportion of males in the LTBI group ($n = 9$[41,43,45–48,50,53,54]) ranged from 30.4%[47] to 64.7%[45].

Sensitivity of QFT-Plus for active TB diagnosis, as a surrogate for LTBI diagnostic sensitivity

Among those with active TB, the pooled proportion of diagnostic sensitivity of QFT-Plus for active TB was 0.94 (95% CI = 0.90–0.98; $I^2 = 75.8\%$, 95% CI = 27.3%–87.5%)[44,48,49,51–53] (Fig. 2). The pooled proportion of diagnostic sensitivity of QFT-Plus-TB1 and QFT-Plus-TB2 for active TB was 0.91 (95% CI= 0.79–0.98; $I^2 = 88.5\%$, 95% CI = 68.7%–93.7%)[44,48,49,53] and 0.95 (95% CI = 0.88–0.99; $I^2 = 80.2\%$, 95% CI = 18.1%–90.7%)[44,48,49,53] (Figs. 3 and 4), respectively.

Among those classified as LTBI based on another immune diagnostic test, the diagnostic sensitivity of QFT-Plus for LTBI was 0.91 (95% CI = 0.84–0.96; $I^2 = 85.4\%$, 95% CI = 75.1%–90.2%)[41,43–48,50,51,53,54] (Fig. 5); the diagnostic pooled specificity of QFT-Plus for LTBI was 0.95 (95% CI = 0.93–0.97; $I^2 = 75.5\%$, 95% CI = 44.9%–85.7%)[41,43–47,50,51,54] (Fig. 6).

Specificity of the QFT-Plus for active TB diagnosis, as a surrogate for LTBI diagnostic specificity

The diagnostic pooled specificity of QFT-Plus for healthy status was 0.96 (95% CI = 0.93–0.99; $I^2 = 76.8\%$, 95%

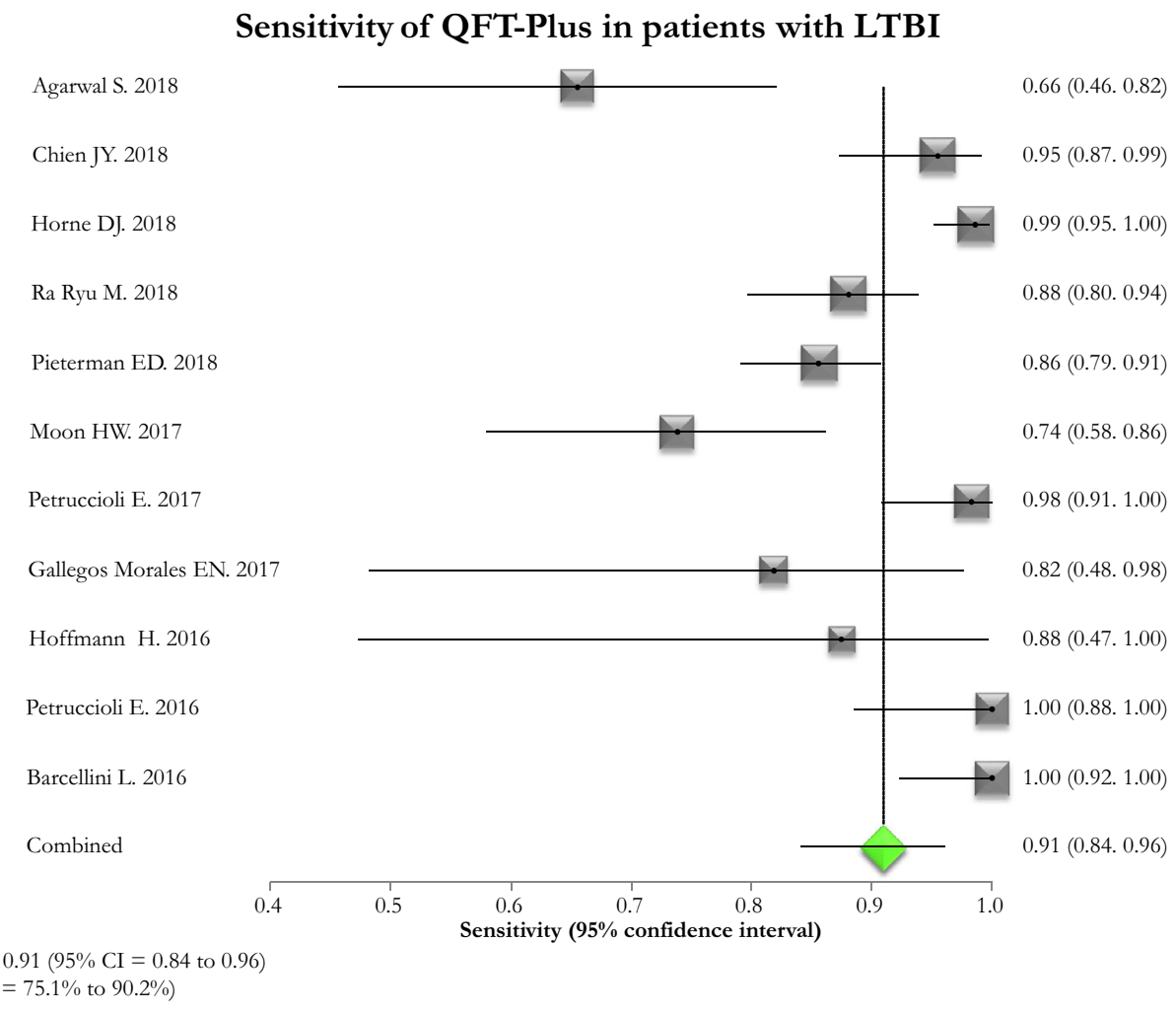


Fig. 5. Sensitivity of QFT-Plus in patients with LTBI.

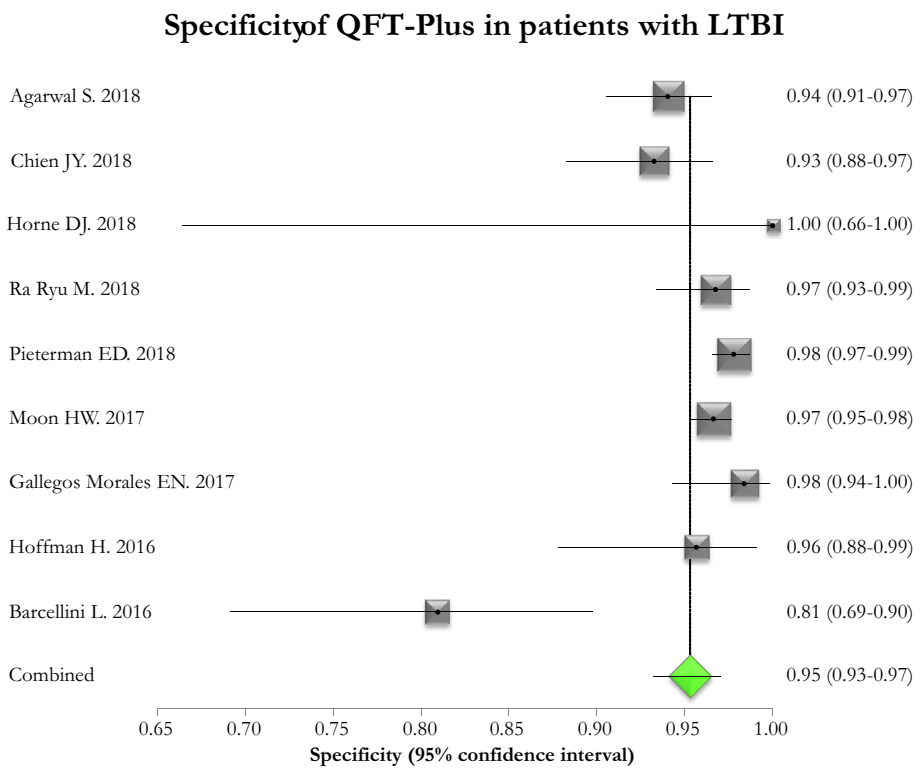
CI = 40.4%–87.3%)[40,42,48,49,51,52,54] (Fig. 7). Only four studies described sensitivity and specificity in at-risk categories (i.e., health care workers-HCWs[41,42,47,51]; data not shown). The risk of bias assessed by QUADAS-2 was low (<60%) in most of the cases and for all four domains (Fig. 8).

Discussion

To the best of our knowledge, this is the first systematic review and meta-analysis on QFT-Plus, comparing its diagnostic performance with previous immunological tests. We showed that the sensitivity of the QFT-Plus for active TB diagnosis is higher if compared with that reported by QFT-GIT.[28] Although the number of scientific studies, whose primary research question is focused on the above-mentioned comparison, is limited and, therefore, the diagnostic accuracy might change in the near future following the implementation of large-sized studies, it is important to highlight that the higher sensitivity is due to the responses to TB2 tube containing both CD4 and CD8 peptides. Numerous studies on CD8 T cell response have inspired the development of QFT-Plus assay. In the last years, CD8 T cell response has been associated to an increased mycobacterial load in active TB[30,38] and to recent Mtb exposure.[35] Moreover, increased CD8 T cell response has been detected in active TB patients with HIV infection[55] and in young children with TB disease.[56] This highlights the importance of the ex vivo inclusion of short peptides inducing CD8 T-cell responses, to improve the sensitivity of the test.

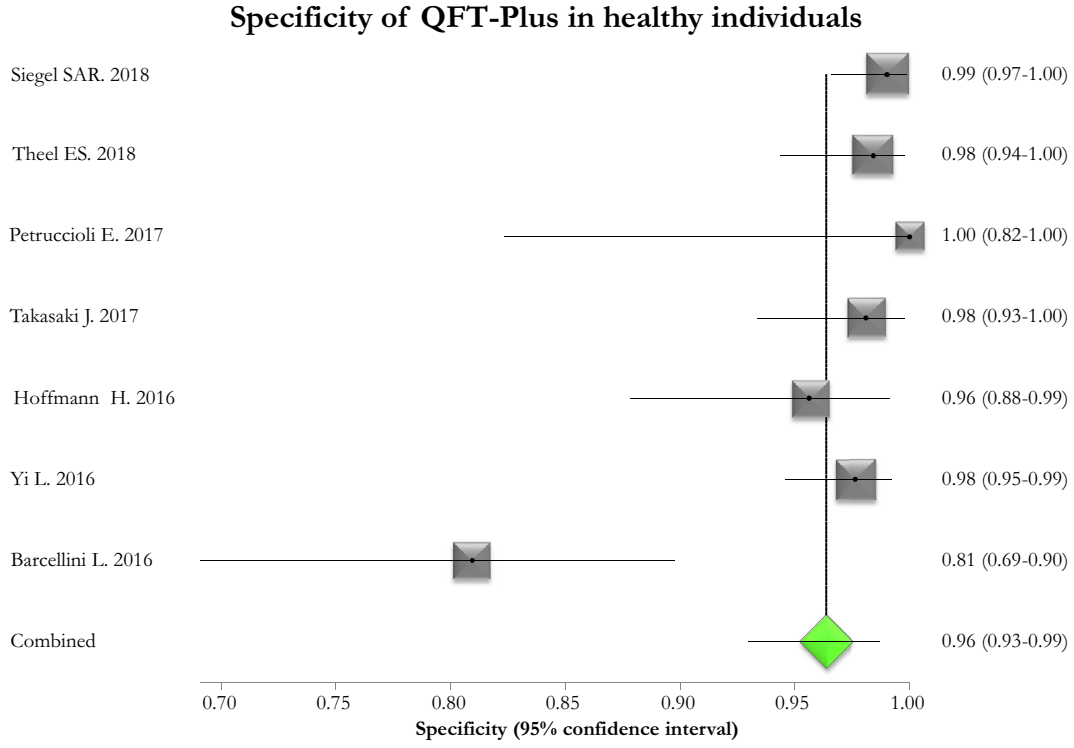
The inclusion of TB2 peptides increased the sensitivity of QFT-Plus assay without affecting the specificity of the test. The specificity has been evaluated in subjects not exposed to TB, as students,[54] healthy donors chosen according to epidemiological criteria,[40,52] healthy donors scored negative to QFT-in tube assay[48]; HCWs in which TST was not done[51] or resulted negative to TST[42] or in patients with diseases different from active TB, as NTM pulmonary disease.[40] To overcome the difficulty to evaluate the accuracy of tests for LTBI diagnosis, some studies used selected population groups with presumed LTBI based on QFT-GIT[48,53] or TST positivity.[41,54]

Several limitations can be found in the selected studies; in particular, the epidemiologic study design was observational and with a different analytical approaches (e.g., case-control or cross-sectional). Furthermore, not all studies evaluated TB and LTBI cases and pooling was not always possible. Although we could not assess the diagnostic performance in some specific categories (e.g., HIV-infected or other immunocompromised patients), it is possible that TB2 peptides could improve the sensitivity in immunocompromised subjects. Unfortunately, the effect of HIV infection on QFT-plus results was investigated only in two studies, not included in the meta-analysis for methodological reasons.[65,66] Studies based on the comparison of different experimental immunological techniques have not been carried out. A comprehensive assessment of



Random effects:
Pooled proportion= 0.95 (95% CI = 0.93 to 0.97)
I^2= 75.5% (95% CI = 44.9% to 85.7%)

Fig. 6. Specificity of QFT-Plus in patients with LTBI.



Random effects:
Pooled proportion= 0.96 (95% CI = 0.93 to 0.99)
I^2= 76.8% (95% CI = 40.4% to 87.3%)

Fig. 7. Specificity of QFT-Plus in healthy individuals.

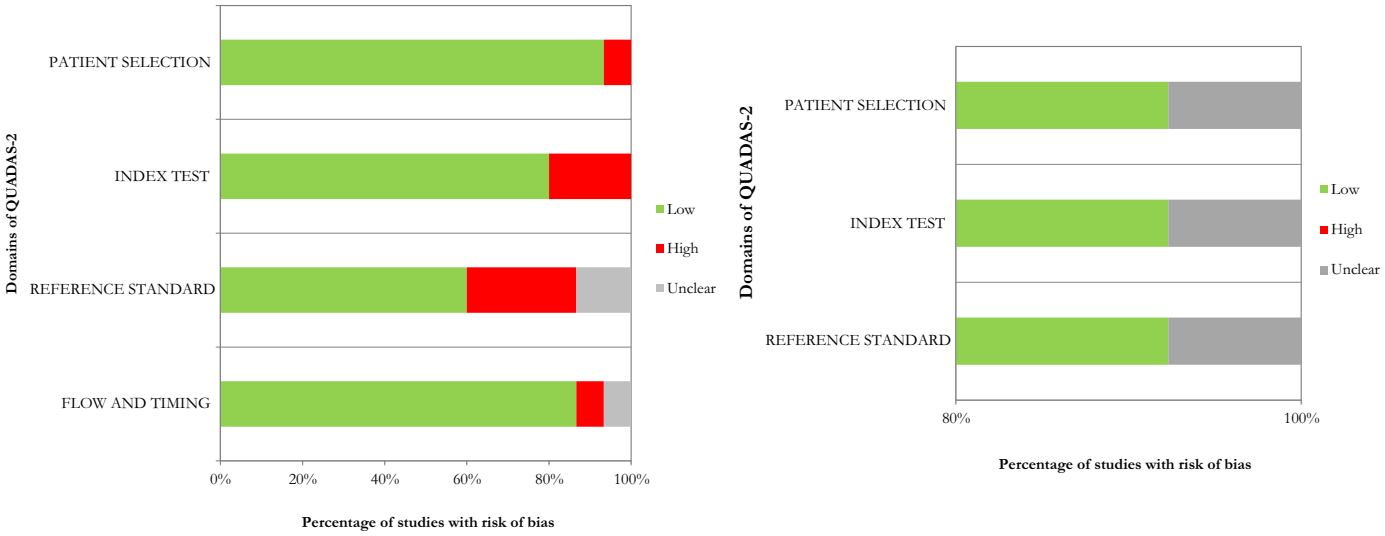


Fig. 8. QUADAS-2 of the selected studies.

the benefit of the current diagnostic options in different population groups, such as those at highest risk of developing TB, could allow policymakers and physicians to identify the best approaches.[63,64] Moreover, the diagnostic performance in extra-pulmonary TB cases has been poorly carried out. A systematic review and meta-analysis of Sester[28] showed interesting findings on the detection of extra-pulmonary forms, which deserve to be investigated using the new QFT plus. The absence of a gold standard for the identification of healthy individuals was associated with the recruitment of low-risk population groups; a more careful and homogeneous methodological approach, based on the identification of a clear comparator, might help the statistical comparison of different studies in the future.

In conclusion, this meta-analysis showed for the first time that the sensitivity of the QFT-Plus for detecting Mtb infection is higher in comparison with that estimated for QFT-GIT. The higher sensitivity can be associated with responses to TB2 containing both CD4 and CD8 peptides. Further and larger studies are needed to confirm these findings.

Declaration of interests

DG received consultant fees for public speaking in international meetings by Qiagen.

SA reports grants and personal fees from Bayer Healthcare, grants and personal fees from Aradigm Corporation, grants and personal fees from Grifols, personal fees from Astra Zeneca, personal fees from Basilea, personal fees from Zambon, personal fees from Novartis, personal fees from Raptor, grants and personal fees from Chiesi, personal fees from Actavis UK Ltd, personal fees from Horizon, grants and personal fees from INSMED, outside the submitted work.

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