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Evaluation of cytokines as a biomarker to distinguish active tuberculosis from latent tuberculosis infection: a diagnostic meta-analysis

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ABSTRACT

Objectives With a marginally effective vaccine and no significant breakthroughs in new treatments, a sensitive and specific method to distinguish active tuberculosis from latent tuberculosis infection (LTBI) would allow for early diagnosis and limit the spread of the pathogen. The analysis of multiple cytokine profiles provides the possibility to differentiate the two diseases.

Design Systematic review and meta-analysis.

Data sources PubMed, Cochrane Library, Clinical Key and EMBASE databases were searched on 31 December 2019.

Eligibility criteria We included case–control studies, cohort studies and randomised controlled trials considering IFN-γ, TNF-α, IP-10, IL-2, IL-10, IL-12 and VEGF as biomarkers to distinguish active tuberculosis and LTBI.

Data extraction and synthesis Two students independently extracted data and assessed the risk of bias. Diagnostic OR, sensitivity, specificity, positive and negative likelihood ratios and area under the curve (AUC) together with 95% CI were used to estimate the diagnostic value.

Results Of 1315 records identified, 14 studies were considered eligible. IL-2 had the highest sensitivity (0.84, 95% CI: 0.72 to 0.92), while VEGF had the highest specificity (0.87, 95% CI: 0.73 to 0.94). The highest AUC was observed for VEGF (0.85, 95% CI: 0.81 to 0.88), followed by IFN-γ (0.84, 95% CI: 0.80 to 0.87) and IL-2 (0.84, 95% CI: 0.81 to 0.87).

Conclusion Cytokines, such as IL-2, IFN-γ and VEGF, can be utilised as promising biomarkers to distinguish active tuberculosis from LTBI.

PROSPERO registration number CRD42020170725.

Strengths and limitations of this study

- ► All stages of the study were conducted by two researchers independently and supervised by a third reviewer.
- ► This study was performed with the methods of the Cochrane Handbook for Systematic Reviews of Interventions and provided evidence regarding the diagnostic value of cytokines in the differentiation of active tuberculosis and latent tuberculosis infection.
- ► The heterogeneity was relatively high. Study design, reference standard and cytokine determination method were the primary sources of heterogeneity.

INTRODUCTION

Tuberculosis is caused by *Mycobacterium tuberculosis* that often affects the lungs. Globally, an estimated 10.0 million people fell ill with tuberculosis in 2018, a number that has been relatively stable in recent years.[1] Coinfection with tuberculosis and AIDS,[2] tuberculosis and diabetes,[3] liver damage caused by antituberculosis drugs[4] and ambient air pollution[5] are all huge obstacles to achieve the 'End Tuberculosis Goal'. According to the WHO, the number of persons with both incident and prevalent tuberculosis remains the highest in the South-East Asian and African regions.[6]

It is estimated that approximately 1.7 billion individuals in the world are latently infected with *M. tuberculosis*.[7] Among them, 5%–10% will develop active tuberculosis (ATB) during their lifetime, especially when their immune system is weak. On the country level, China and India had the highest latent tuberculosis infection (LTBI) burden, followed by Indonesia.[7] With reasonable assumptions for reactivation risks, incident tuberculosis cases arising from the LTBI reservoir would prohibit reaching the 'End Tuberculosis Strategy' goal. Accurate and rapid diagnosis would allow the medications to be allocated appropriately, and actions can be taken to curtail *M. tuberculosis* spread more effectively. The traditional tuberculin skin test (TST) and the recently developed interferon-gamma release assay (IGRA) can assist in the diagnosis of LTBI, but they neither distinguish between infection and active disease nor predict the risk of activation from latent infection.[8–10]

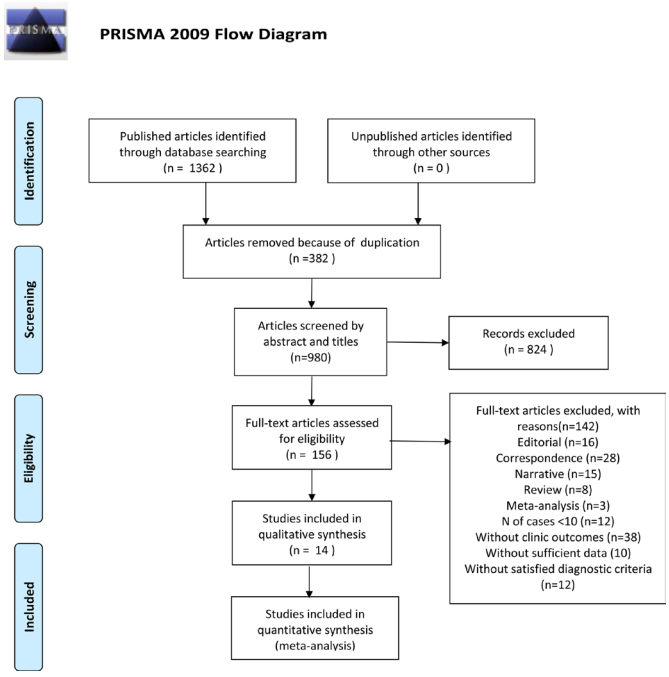

Figure 1 Flow diagram of the search process. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

IGRAs are blood tests that detect the secretion of IFN-γ by sampled lymphocytes after stimulation with proteins that are relatively specific for *M. tuberculosis*.[11] As IFN-γ is produced by memory T cells,[12] it is not surprising that the measurement of this cytokine alone cannot accurately distinguish LTBI subjects from those with active disease.[13] Detecting other cytokines and adopting separate or combined methods can significantly improve diagnostic accuracy. With a marginally effective vaccine and no apparent breakthrough in new treatments, a sensitive and specific method to distinguish the active disease from LTBI would allow for an early diagnosis and limit the spread of the pathogen. Thus, we performed this meta-analysis through an extensive and in-depth search for relevant studies to analyse the possibility of multiple cytokine profiles to differentiate these two diseases.

METHODS

Design

Our protocol was performed using the methods of the Cochrane Handbook for Systematic Reviews of Interventions. We performed this meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[14]

Data sources and searches

We selected PubMed, Cochrane Library, Clinical Key databases and EMBASE for systematic and comprehensive searches. Articles published on 31 December 2019 were searched. The primary search process had no language restrictions. We further read the references cited in the selected articles to identify other relevant studies and improve the search sensitivity. The search terms are listed in online supplementary table S1.

Study selection

We selected articles describing pathological changes of cytokines, including IFN-γ, TNF-α, IP-10, IL-2, IL-10, IL-12 and VEGF, stimulated by *M. tuberculosis* antigen, among patients with ATB and LTBI. Cytokines were analysed quantitatively or qualitatively. The ability of cytokines as biomarkers to discriminate ATB from LTBI was evaluated. We included articles using the designs of either case–control studies, cohort studies or randomised controlled trials (RCTs). The exclusion criteria were as follows: editorial, correspondence, narrative review or system review; the number of ATB or LTBI cases was less than 10; studies did not report any follow-up outcomes and studies did not report true positive (TP), false positive (FP), false negative (FN) and true negative (TN) or did not provide sufficient data to calculate them. Two researchers conducted rigorous and independent assessments of the articles. Differences were resolved through negotiation. We did not find any quantitative and qualitative differences between them in the article search and data extraction phase. Their interagreement was 100%.

Data extraction

Two independent extractors extracted the data. We retrieved and read the entire content of the selected articles and extracted data including the first author, publication date, study area, sample size, sample type, reference standard, demographics (age and gender), clinical characteristics (HIV infection, diabetes, liver or kidney injury, drug resistance, previous history of tuberculosis, extrapulmonary tuberculosis and lung cavity), TP, FP, FN and TN. All data were summarised and processed in the form of a feature table.

Risk of bias assessment

We used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) to assess the quality and risk of bias of each study.[15] The items of QUADAS-2 covered the disease spectrum, gold standard, disease progression bias, verification bias, evaluation bias, clinical evaluation bias, pooling bias, trial implementation, case withdrawal and uncertain results. The evaluation results were defined as 'yes', 'no' or 'unclear'.

Outcomes

The sensitivity, specificity, diagnostic OR (DOR), positive likelihood ratio (PLR) and negative likelihood ratio (NLR), together with 95% CI, were used to estimate the diagnostic value of the cytokines.

Statistical analysis

We used Excel 2010 to draw feature tables and STATA V.15 (StataCorp, College Station, Texas, USA) to perform the meta-analysis. The pooled sensitivity, specificity, PLR, NLR, DOR and 95% CI for each cytokine were calculated. A forest plot was drawn to visually show the difference in the point estimates of each study. A summary receiver

Table 1 Baseline characteristics of the studies

Author	Year of publication	Year of study	Country	Design	Disease	N	Age (years)		Gender (male)	BCG
							Mean	Median (range)		
Won	2017	2015	South Korea	Cohort	ATB	36	63.9	73 (15–86)	15	–
					LTBI	15	55.1	52 (36–75)	8	–
Wu	2017	2015	China	Cohort	ATB	25	51	22–85	18	17
					LTBI	36	48	7–76	12	31
Jeong	2015	2010	South Korea	RCT	ATB	33	–	30 (20–63)	19	21
					LTBI	20	–	44 (22–60)	4	18
Clifford	2019	2012	Australia	RCT	ATB	38	–	28 (25–44)	19	22
					LTBI	43	–	26 (24–31)	21	33
Kim	2015	2010	South Korea	RCT	ATB	28	32.1	21–69	8	9
					LTBI	22	46.5	22–69	4	21
Wang	2018	2009	China	Cohort	ATB	28	46	26–55	16	17
					LTBI	34	43	15–62	15	25
Pathakumari	2015	2010	India	RCT	ATB	39	–	19–60	25	–
					LTBI	35	–	21–58	22	–
Hur	2016	2013	South Korea	RCT	ATB	52	43	26–60	29	–
					LTBI	31	45	38–52	20	–
Zhang	2017	2012	China	RCT	ATB	26	37	24–50	23	–
					LTBI	45	34	28–40	14	–
La Manna	2018	2013	Italia	RCT	ATB	27	–	17–82	21	–
					LTBI	32	–	17–84	24	–
You	2016	2012	South Korea	RCT	ATB	40	52.7	36.3–69.1	31	–
					LTBI	40	63.7	49.5–77.9	27	–
Suzukawa	2016	2010	Japan	RCT	ATB	31	37	21–48	18	–
					LTBI	29	42	23–55	12	–
Yao	2017	2016	China	Cohort	ATB	20	–	29 (16–67)	11	8
					LTBI	15	–	38 (20–67)	8	15
Wang	2012	2009	China	RCT	ATB	66	–	45 (16–86)	39	52
					LTBI	73	–	41 (18–83)	35	54

ATB, active tuberculosis; BCG, Bacillus Calmette-Guerin; LTBI, latent tuberculosis infection; RCT, randomised controlled trial.

operating characteristic (SROC) curve was plotted, and the overall diagnostic value of cytokines was displayed by the area under the curve (AUC). The fixed or random-effect model was applied based on the heterogeneity test. If I^2 >50% or p<0.10, we selected the random-effects model; otherwise, we applied the fixed-effects model. Meta-regression analysis was used to explore the causes of heterogeneity. Egger's test and Begg's test were applied to detect possible publication bias.

Patient and public involvement

Patients and public were not involved in this study.

RESULTS

Search results

Preliminary searching yielded 1362 records. Then, we removed 382 duplicated records, 824 irrelevant articles by reading titles and abstracts and 142 irrelevant articles that did not meet the inclusion criteria after reading the full text. Finally, there were 14 articles included in the meta-analysis (figure 1).[8 10 16–27]

Characteristics of the studies

Articles were published during 2012–2019. They were performed in China (5), India (1), Australia (1), South Korea (5), Japan (1) and Italy (1), respectively. Except for Australia and Italy, all countries had a relatively high burden of tuberculosis. The total number of study subjects was 959, including 476 ATB cases and 483 LTBI cases. One study used the T-spot as the reference standard for ATB,[16] while the others applied the *M. tuberculosis* pathogenic test. One study defined LTBI based on positive TST results and close contact with ATB patients for more than 1 month without clinical symptoms,[17] two studies defined

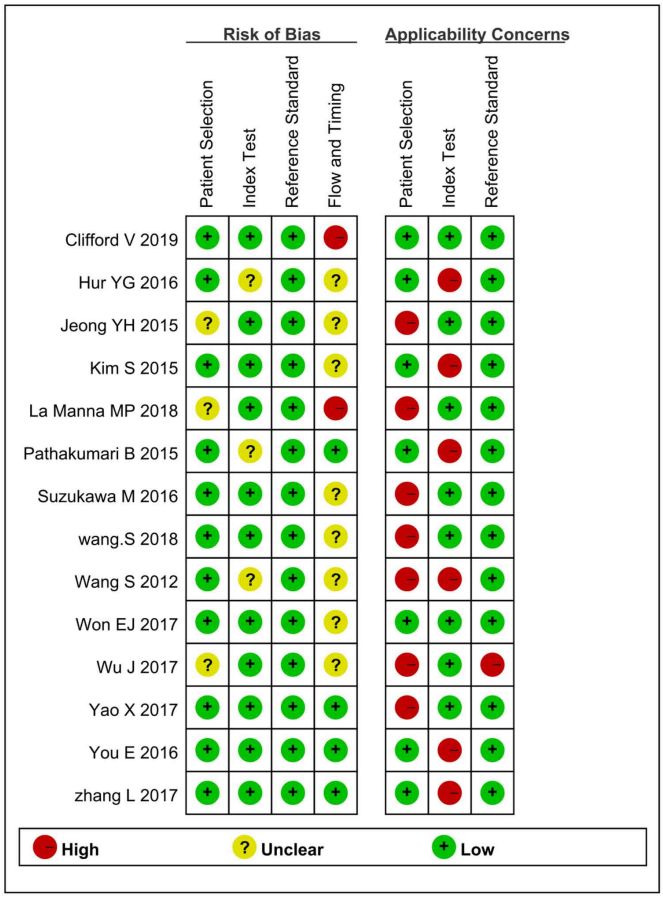

Figure 2 Quality assessment of the studies.

LTBI based on a positive TST and IGRA,[18 19] and the other 11 studies used QuantiFERON-TB Gold In-Tube (QFT-IT), chest X-ray examinations and clinical symptoms as reference standards. Seven studies reported Bacillus Calmette-Guerin vaccination history. Four articles explicitly reported whether the patients had extrapulmonary tuberculosis. The characteristics of the included studies are listed in table 1.

Study quality

As shown in figure 2, two studies had a high risk of bias with flow and timing concerns. We found that the applicability concerns were low for 'patient selection' in seven studies, 'index tests' in six studies, and 'reference standard' in one study.

Pooled diagnostic value of cytokines in distinguishing ATB and LTBI

Seven cytokines, IFN-γ, TNF-α, IP-10, IL-2, IL-10, IL-12 and VEGF, were selected as indicators to calculate the accuracy and ability of their use as biomarkers to differentiate ATB and LTBI. Cytokines and related indicators included in every study are shown in table 2. One study[23] applied the FluoroSpot, five studies[19 21 22 25 27] applied an ELISA assay and eight studies used Luminex to measure the cytokines. The forest plots and SROC curves are shown in online supplementary figures S1–14. The pooled sensitivity, specificity, PLR, NLR, DOR, AUC and heterogeneity index I^2 and p-value are summarised in table 3. The numbers of study subjects in each study are listed in table 4. IL-2 had the highest sensitivity (0.84, 95% CI: 0.72 to 0.92) and VEGF had the highest specificity (0.87, 95% CI: 0.73 to 0.94). IFN-γ had the highest DOR (12, 95% CI: 5 to 26). After drawing the SROC curves for seven cytokines, the highest AUC was 0.85 (95% CI: 0.81 to 0.88) for VEGF, followed by IFN-γ (0.84, 95% CI: 0.80 to 0.87) and IL-2 (0.84, 95% CI: 0.81 to 0.87).

Meta-regression analysis

The meta-regression analysis results are shown in online supplementary tables S2–8 and figures S15–21. Regression models included joint models and models for sensitivity and specificity that were independently established. We identified five factors that may have caused the heterogeneity, including study design, inclusion and exclusion of study subjects, reference standard, independence of the index test and reference standard and the method of the index test.

Publication bias evaluation

Publication bias was judged by Egger's and Begg's test and is shown in online supplementary table S9. IP-10 had an apparent publication bias (Egger's test p=0.078; Begg's test p=0.016). The other six cytokines did not show a significant publication bias. The funnel plots are illustrated in online supplementary figures S22–28.

DISCUSSION

The advantage and originality of this meta-analysis lay in its search of major databases, considering as many cytokines as possible, and including various types of professional studies. We evaluated seven cytokines (IFN-γ, TNF-α, IP-10, IL-2, IL- 10, IL-12 and VEGF) in the scope of the meta-analysis and probed their capacity as biomarkers to distinguish ATB and LTBI, which is unprecedented in previous studies. We observed that IL-2 had the highest sensitivity, and VEGF had the highest specificity. Although the alternative test using smear microscopy suggested a sensitivity of at least 80% and a specificity of at least 98%,[28] cytokines such as IL-2 and VEGF also have potential discrimination abilities. As expected, IFN-γ had the highest DOR value.

To explore factors that may cause heterogeneity and bias in this meta-analysis, we first stratified the articles by the study design. Except for four studies using cohort or case–control designs,[8 16 20 26] the other 10 studies were RCTs. The RCT has distinct advantages and can effectively prevent selective bias. Then, we performed a subgroup analysis by the reference standard. Although TST and IGRA are commonly used as screening tools, there is no unified and clear reference standard for LTBI. In this meta-analysis, one study defined LTBI based on TST,[17] two studies comprehensively considered the results of TST and IGRA,[18 19] and the other 11 studies relied on IGRA to determine *M. tuberculosis* infection.

Table 2 Cytokines and related indicators included in every study

Author	Cytokine	Reference test	Diagnostic test	TP	FN	FP	TN	Cut-off value (pg/mL)
Won	TNF-α	IGRA	Luminex	21	15	1	14	373.6
	IL-10			23	13	3	12	0.145
Wu	IFN-γ	IGRA	Luminex	13	12	4	32	1600
	TNF-α			20	5	17	19	1576
	IL-2			21	4	15	21	976.3
	IL-10			20	5	15	21	251
	IP-10			19	6	12	24	1139
Jeong	IFN-γ	TST	Luminex	18	2	8	25	172.84
	IP-10			17	3	3	30	23 780
Clifford	IFN-γ	TST and IGRA	Luminex	34	4	9	34	1215
	TNF-α			28	10	4	39	332
	IP-10			16	22	4	39	19301
	IL-2			33	5	8	35	398
Kim	IFN-γ	TST and IGRA	ELISA	20	8	6	16	None
	IP-10			28	0	18	4	None
	TNF-α			27	1	12	10	None
Wang	IFN-γ	IGRA	Luminex	18	10	8	26	77.6
	IP-10			13	15	3	31	10821
	IL-12			15	13	9	25	57.39
	VEGF			15	13	3	31	225.1
Pathakumari	IFN-γ	IGRA	ELISA	8	31	5	30	116.4
	TNF-α			21	18	5	30	381.8
	IL-12			15	24	5	30	171.4
Hur	TNF-α	IGRA	ELISA	38	14	9	22	302.2
Zhang	IFN-γ	IGRA	FluoroSpot	24	2	9	36	248
La Manna	IFN-γ	IGRA	Luminex	19	8	3	29	124
	IP-10			22	5	5	27	637
	IL-2			22	5	2	30	90
	IL-12			15	12	4	28	6
You	IP-10	IGRA	ELISA	29	11	12	28	1587.76
	IL-2			23	17	16	24	106.51
	IL-10			34	6	19	21	0.18
Suzukawa	TNF-α	IGRA	Luminex	10	21	2	27	660.6
	IP-10			23	8	14	15	33082
	IL-2			30	1	22	7	333.2
	IL-10			20	11	3	26	0.8
	IL-12			17	14	6	23	10.3
	VEGF			8	23	2	27	23.4
Yao	IP-10	IGRA	Luminex	10	10	1	14	1580
	VEGF			17	3	4	11	37.54
Wang	IP-10	IGRA	ELISA	59	7	42	31	451.3
	IL-2			57	9	29	44	13.1

FN, false negative; FP, false positive; IGRA, interferon-gamma release assay; TN, true negative; TP, true positive; TST, tuberculin skin test.

Table 3 Summary of the meta-analysis for each cytokine

Cytokines	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	Heterogeneity of sensitivity (I^2 p)	Heterogeneity of specificity (I^2 p)	AUC (95% CI)
IFN-γ	0.72 (0.52 to 0.86)	0.82 (0.76 to 0.86)	4.0 (3.0 to 5.3)	0.34 (0.19 to 0.62)	12 (5 to 26)	88.97%, <0.01	0%, 0.50	0.84 (0.80 to 0.87)
TNF-α	0.70 (0.56 to 0.82)	0.79 (0.64 to 0.89)	3.4 (2.2 to 5.3)	0.37 (0.26 to 0.53)	9 (6 to 14)	81.34%, <0.01	80.81%, <0.01	0.81 (0.78 to 0.85)
IP-10	0.75 (0.60 to 0.86)	0.74 (0.56 to 0.87)	2.9 (1.8 to 4.7)	0.34 (0.24 to 0.49)	9 (5 to 14)	84.34%, <0.01	89.61%, <0.01	0.81 (0.77 to 0.84)
IL-2	0.84 (0.72 to 0.92)	0.66 (0.44 to 0.82)	2.5 (1.4 to 4.3)	0.24 (0.13 to 0.43)	10 (4 to 26)	79.36%, <0.01	87.31%, <0.01	0.84 (0.81 to 0.87)
IL-10	0.74 (0.62 to 0.84)	0.72 (0.52 to 0.86)	2.6 (1.5 to 4.5)	0.36 (0.25 to 0.51)	7 (4 to 15)	51.98%, 0.10	76.62%, 0.01	0.79 (0.75 to 0.83)
IL-12	0.50 (0.41 to 0.59)	0.82 (0.74 to 0.87)	2.7 (1.8 to 4.0)	0.62 (0.51 to 0.75)	4 (2 to 8)	0%, 0.42	0%, 0.44	0.72 (0.68 to 0.76)
VEGF	0.59 (0.35 to 0.80)	0.87 (0.73 to 0.94)	4.5 (2.5 to 8.0)	0.47 (0.27 to 0.80)	10 (4 to 22)	85.80%, <0.01	42.08%, 0.16	0.85 (0.81 to 0.88)

PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic OR; AUC, area under the curve.;

In addition to the study design and reference standard, cytokine detection methods may also affect the results. For 14 studies included in this meta-analysis, one study used FluoroSpot,[23] five studies used traditional ELISA or capillary-based ELISA[19 21 22 25 27] and the other eight studies used Luminex. The FluoroSpot applies selective filters for emission, which can analyse each analyte separately and then identify the double-stained and triple-stained spots. It can detect two or three cytokines at the same time with high sensitivity and specificity.[29] ELISA is widely used in the determination of cytokines in various body fluids with high repeatability. However, traditional ELISA has the disadvantages of complicated operation, long measurement time and large sample consumption. Capillary-based ELISA significantly improves the above disadvantages, shortening the measurement time to 16 min and reducing the sample volume to 20 µL.[30] Luminex is now a vital tool for the quantitative determination of cytokines. It is possible to measure multiple cytokines simultaneously with a small sample in a short time by using hundreds of micrometer-scale specially prepared microspheres.[31] Also, the precision of the equipment used to measure the cytokines and the choice of cytokine threshold would affect the diagnostic value. In most cases, the threshold is determined by the receiver operating characteristic curve with maximised sensitivity and specificity.[32 33] However, in areas with a low burden of tuberculosis, the threshold may be set at a lower level in order to better distinguish the active and latent tuberculosis.[34]

To improve the diagnostic value, multiple cytokines are usually used in combination. Won *et al* found that a combination of five biomarkers (IL-5, IL-10, TNF-α, VEGF and IL-2/IFN-γ) can predict 95.5% of ATB and 93.3% of LTBI.[8] In another study, the combination of ESAT-6/CFP-10-specific EGF and Rv2032-specific VEGF correctly discriminated against all participants (100%).[35] Kim *et al* reported that the combination of IFN-γ, TNF-α and IL-2R had a sensitivity of 100% and a specificity of 86.36%.[19] Wang *et al* found that six cytokines in combination (tuberculosis antigen-stimulated IFN-γ, IP-10 and IL-1Ra; unstimulated cytokines of IP-10, VEGF and IL-12) had a sensitivity of 85.7% and a specificity of 91.3%.[20] Our analysis showed that the combination of cytokines represented by IL-2, VEGF and IFN has potential value in screening for patients with ATB and LTBI. However, the immune response to *M. tuberculosis* infection is complex and multifaceted. The impact of coinfection with HIV and other iatrogenic causes on test performance in immunocompromised patients needs to be determined to understand the full benefits and limitations of this technology.

Millions of patients with LTBI are underdiagnosed every year,[36 37] and there is an urgent need for better diagnostic tools.[38] The quick differentiation and correct identification of ATB from LTBI is the current focus of global tuberculosis prevention and control. Blood and urine are good sources of samples for diagnosis without causing harm to the human body.[39] Findings from our meta-analysis have particular guiding significance and a

Table 4 The number of subjects in each study

Cytokines	Number of participants in each study	Number of studies
IFN-γ	Wu[16] (71), Jeong[17] (53), Clifford[18] (81), Kim[19] (50), Wang[20] (62), Pathakumari[21] (74), Zhang[23] (71), La Manna[24] (59)	8
TNF-α	Won[8] (51), Suzukawa[10] (60), Wu[16] (71), Clifford[18] (81), Kim[19] (50), Pathakumari[21] (74), Hur[22] (83)	7
IP-10	Suzukawa[10] (60), Wu[16] (71), Jeong[17] (53), Clifford[18] (81), Kim[19] (50), Wang[20] (62), La Manna[24] (59), You[25] (80), Yao[26] (35), Wang[27] (139)	10
IL-2	Suzukawa[10] (60), Wu[16] (71), Clifford[18] (81), La Manna[24] (59), You [25] (80), Wang[27] (139)	6
IL-10	Won[8] (51), Suzukawa[10] (60), Wu[16] (71), You[25] (80)	4
IL-12	Suzukawa[10] (60), Wang[20] (62), Pathakumari[21] (74), La Manna[24] (59)	4
VEGF	Won[8] (51), Suzukawa[10] (60), Wang[20] (62), Yao[26] (35)	4

theoretical basis for clinical practice, which could provide clues for developing new methods and techniques to screen for tuberculosis and LTBI.

Our study has several limitations. First, as mentioned above, the differences in study design, reference standards and cytokine determination method may be sources of bias. Second, the studies involved in the analysis were mainly conducted in countries with a high burden of tuberculosis. The diagnostic value of cytokines in low prevalence areas is uncertain. Third, there are differences in the quality of different research groups, which may also contribute to heterogeneity. Although we used QUADAS-2 to assess the quality and risk of bias of each study, it could not fully consider all kinds of causes of bias and heterogeneity.

Although this meta-analysis has several limitations mentioned above, the findings of this study are valuable and provide evidence regarding cytokines, such as IL-2, IFN-γ and VEGF, to be utilised as promising biomarkers to distinguish ATB from LTBI.

Contributors BQ and JW conceived, initiated and led the study. BQ, QL, ZL, HS, DX, YeJ, YaJ and DT collected the data. BQ and QL analysed the data with input from all of the authors. BQ and JW prepared the manuscript. All authors reviewed and approved the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The ethical approval can be exempted as this is a systematic review and meta-analysis of data from already published studies.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. All data generated or analysed during this study are included in this published article.

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