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Adjunctive therapy with V-5 Immunitor (V5) for the treatment of tuberculosis patients: a meta-analysis

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Objective: To evaluate the effectiveness and safety of V-5 immunitor (V5) added to chemotherapy of tuberculosis (TB) patients. *Methods*: The databases Medline, Embase, Biosis, Cochrane Central Register of Controlled Trials, SCI, CBM, VIP and CNKI were searched. Randomized controlled trials (RCT) and controlled clinical trials (CCT) of V5 immunitor with or without a placebo-control as adjuvant therapy in the chemotherapy of TB patients were included. Two reviewers independently performed data extraction and quality assessment. Data were analyzed using RevMan 5.3 software by The Cochrane Collaboration. *Results*: Four studies were included. At the end of the follow-up period, pooled RR (Risk Ratio) and its 95% CI of sputum smear conversion rate were 4.91 (3.32, 7.28) in drug-sensitive, drug-resistant TB patients or HIV-TB co-infection patients. When analyzing inflammation biomarkers including ESR and leukocyte accounts, pooled mean difference and its 95% CI of ESR and leukocyte accounts were -7.62 (-9.55, -5.68) and -2.13 (-2.58, -1.68), respectively. As to body weight, pooled mean difference and its 95% CI were 0.96 (-1.13, 3.05) in TB patients. Two clinical trials were included for analyzing temperature after using V5 immunitor, pooled mean difference and its 95% CI were -0.34 (-0.46, -0.22) in TB patients. These results suggested that V5 immunitor holds important promise in improving sputum conversion to AFB- and inhibiting inflammatory reaction in TB patients, but showed no significant promotion to the increase in body weight based on this meta-analysis. Compared with the control group, V5 immunitor may have some potential in decreasing the temperature of TB patients. No systemic adverse events were reported. *Conclusion*: Added to chemotherapy, V5 immunitor seems to be helpful in the treatment of TB patients in terms of improving sputum conversion and reducing inflammatory reactions.

1. Introduction

It is widely accepted that tuberculosis (TB) has become an increasingly serious global public health problem as a major cause of illness and death. Especially the emergence of multidrug-resistant TB (MDR-TB) or even extensively drug-resistant TB (XDR-TB) exacerbated this problem (Cohen et al. 2014; Jenkins et al. 2013; Lienhardt et al. 2010; Marks et al. 2014; Naidoo et al. 2015; Wingfield et al. 2014). Still, an ideal forms of chemotherapy in not available for the treatment of TB. Some side effects including poor patient adherence, treatment failure, and the emergence of drug resistance are widespread under chemotherapy with isoniazid, rifampicin, pyrazinamide and ethambutol (Lange et al. 2014; Wells et al. 2011; Yew et al. 2011). Even short-course chemotherapy still requires 6 months with an intensive phase in the first 2 months and further 4 months of continuation phase. Thus, it is generally accepted that immunotherapy combined with anti-tubercular chemotherapy has an important potential in improving treatment outcomes (Efremenko et al. 2013; Hawn et al. 2014; World Health Organization 2007; Sharma et al. 2012; Tye et al. 2015).

The oral therapeutic vaccine V5 immunitor was originally used to treat chronic hepatitis B and C (Batdelger et al. 2008, 2009). One clinical trial reported that V5 was found to produce mycobacterial clearance in sputum smears of 94.4% of patients with pulmonary TB, hepatitis C and HIV-co-infection (Arjanova et al. 2010). A placebo-controlled, randomized trial was conducted to confirm that V5 was safe and effective for treating TB (Arjanova et al. 2011). In this paper, we explore the effect and safety of V5 immunitor as adjuvant therapy for TB patients in a meta-analysis of available study results.

2. Investigations and results

2.1. Description of studies and quality assessment

The initial search extracted 669 articles, and after selection, 4 met the inclusion criteria (Fig. 1).

Table 1 shows the characteristics of the four included studies (Arjanova et al. 2012a; Arjanova et al. 2011; Butov et al. 2012; Butov et al. 2011), they included subjects with HIV (Arjanova et al. 2012; Arjanova et al.

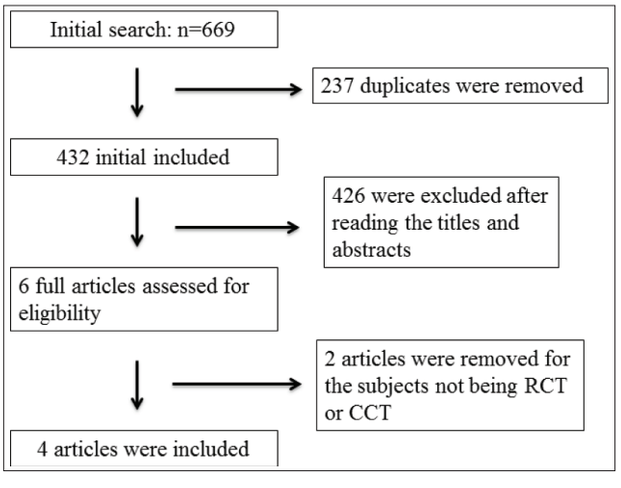

Fig. 1: Flow diagram of study searching and selection process.

2011; Butov et al. 2012). In these trials, a single daily dose of V5 immunitor was used as adjuvant therapy for TB patients. All patients received either standard TB therapy consisting of orally administered isoniazid (H; 300 mg), rifampicin (R; 600 mg), pyrazinamide (Z; 2000 mg), ethambutol (E; 1200 mg) and an intramuscular injection of streptomycin (S; 1000 mg) or individualized treatment regimens as decided by the clinicians based on results of drug resistance testing or prior medical history. The third category of patients obtained palliative treatment consisting of two drugs, H and R (Table 1).

Table 2 shows the quality assessment of the included studies. After contacting the author, 2 articles got ''yes'' in ''adequate sequence generation''(Butov et al. 2012). The results at all follow-up time points which were pre-specified were fully reported in included studies. Three out of four trials demonstrated the time point of 1 month for the results of interest (Arjanova et al. 2011; Butov et al. 2011, 2012) , but one trial reported 3 months for the experimental group and 3.5 months for the control group (Arjanova et al. 2012a).

2.2 Primary outcomes of intervention

2.2.1. Sputum smear conversion rates of TB patients

Figure 2 shows the conversion rates of sputum smears to acid-fast bacilli negative (AFB-) of the V5 immunitor recipient groups compared with the control groups receiving chemotherapy, with or without a placebo injection. This study set the length of 3.5 months as a short course for treating TB patients. One clinical trial reported that the conversion rate of V5 recipients in drug-sensitive, drug-resistant TB patients or HIV–TB co-infection patients was similar (Butov et al. 2012). Another study demonstrated that the conversion rate of V5 recipients with MDR-TB patients seemed to be higher than in first-diagnosed TB patients, but the difference was not significant (P = 0.62) (Butov et al. 2011). Thus, we took the conversion rate of drug-sensitive, drug-resistant TB patients and HIV–TB co-infection patients together for data analysis. The fixed effect model was applied at the end of the follow-up period (1, 3 or 3.5 months after the first V5 immunitor administration), the pooled RR and its 95% CI was 4.91 (3.32, 7.28), with statistical significance (P<0.00001).

Figure 3 shows a funnel plot for studies reporting the RRs of the conversion rates of sputum smears to AFB- as a measure of treatment effect. The plot is not very symmetrical, and all studies fall within the 95% CI axis for a given standard error. There may be few studies missing from the search strategy, and so there is minimal evidence of publication bias.

2.3. Secondary outcomes

2.3.1. Effect on inflammation

As mentioned before, the conversion rates of sputum smears to AFB- was similar in AFB- drug-sensitive, drug-resistant TB patients or HIV–TB co-infection patients. Thus, we took these TB patients together for

Table 1: Characteristics of included studies

NO.	Author	Experimental group					Control group				Follow-up time
		Number	Age	Combined disease	Anti-tuberculosis treatment regimen	V5 doses	Number	Age	Combined disease	Anti-tuberculosis treatment regimen	
1	Butov 2012 (Butov et al. 2012)	71	39.6±10.9	HIV(10)	Std, Ind or Pal	once-daily pill	61	39.1±11.8	HIV(7)	Std, Ind or Pal	1 month
2	Arjanova 2012 (Arjanova et al. 2012)	36	37.3±10.8	HIV (1)	HR	once-daily pill	36	42.9±11.8	HHIV (2)	HR	3 / 3.5 months
3	Butov 2011 (Butov et al. 2011)	24	40.8 ± 12	-	Std or Ind	once-daily pill	10	34.8±3.58	-	Std or Ind	1 month
4	Arjanova 2011 (Arjanova et al. 2011)	27	38.1 ± 10	HIV(7)	Std or Ind	once-daily pill	28	38.3 ± 12	HIV(10)	Std or Ind	1 month

H: Isoniazid R: Rifampicin. Std: Standard, Ind: Individual, Pal: Palliative.

Table 2: Quality assessment of included studies

NO.	Included studies	Type of study	Adequate sequence generation	Allocation concealment	Blinding	Incomplete outcome data addressed
1	Butov 2012 (Butov et al. 2012)	RCT	Y	U	U	N
2	Arjanova 2012 (Arjanova et al. 2012)	CCT	U	N	N	N
3	Butov 2011 (Butov et al. 2011)	RCT	U	U	U	N
4	Arjanova 2011 (Arjanova et al. 2011)	CCT	Y	U	U	N

RCT: randomized controlled trial, CCT: clinical controlled trial, Y: yes, N: no, U: unclear.

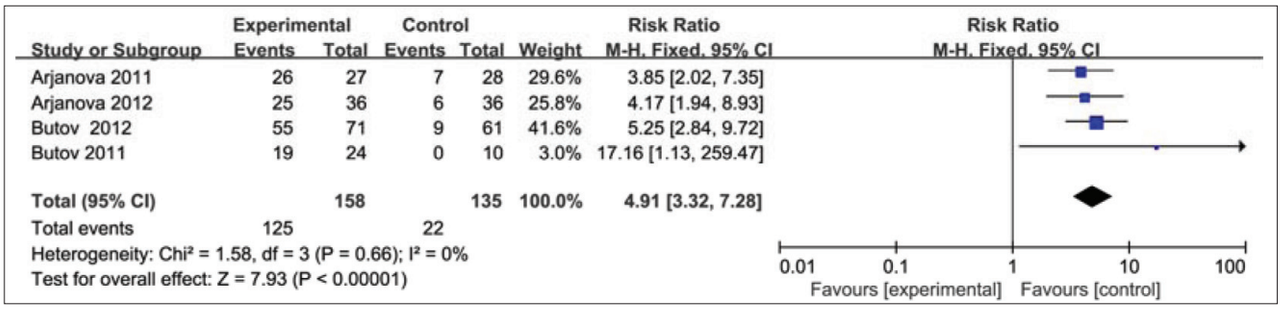

Fig. 2: Meta analysis of sputum smear negative conversion rates in TB patients. Vertical line indicated no difference between the compared two groups (V5 immunitor vs. control). Squares indicated point estimates of risk ratio (RR) in each individual study, the size of the squares indicated the weight of the corresponding study in the meta-analysis, 95%CIs of point estimates were shown by horizontal lines. Pooled RR and its 95%CI are shown by diamond shape.

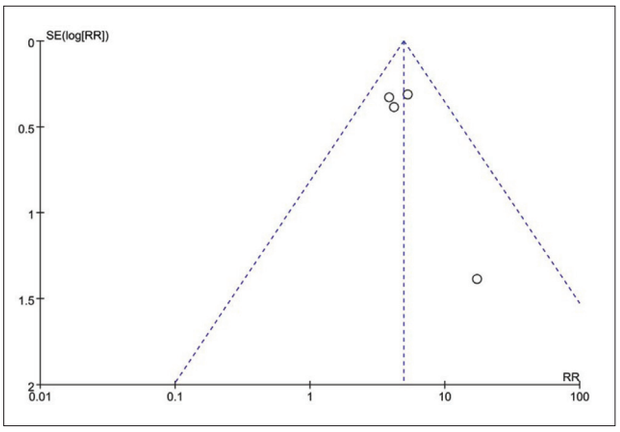

Fig. 3: Funnel plot for the outcome of the conversion rates of sputum smears to AFB-in TB patients.

data analysis of ESR. Figure 4 shows that the fixed effect model was applied at the end of the follow-up period, the pooled mean difference and its 95% CI was -7.62 (-9.55, -5.68), with statistical significance (P<0.00001). And the funnel plot was relatively symmetrical, and all studies fall within the 95% CI axis for a given standard error. There is little evidence of publication bias (Fig. 5).

Leukocyte counts of drug-sensitive, drug-resistant TB patients or HIV–TB co-infection patients were analyzed together. Figure 6 demonstrates that the fixed effect model was applied at the end of the follow-up period, the pooled mean difference and its 95% CI was -2.13 (-2.58, -1.68), with statistical significance (P<0.00001). And the funnel plot was relatively symmetrical, and all studies fall within the 95% CI axis for a given standard error. There was little evidence of publication bias (Fig. 7).

2.3.2. *Effect on body weight*

When analyzing the body weight of all TB patients, the fixed effect model was applied at the end of the follow-up period, the pooled

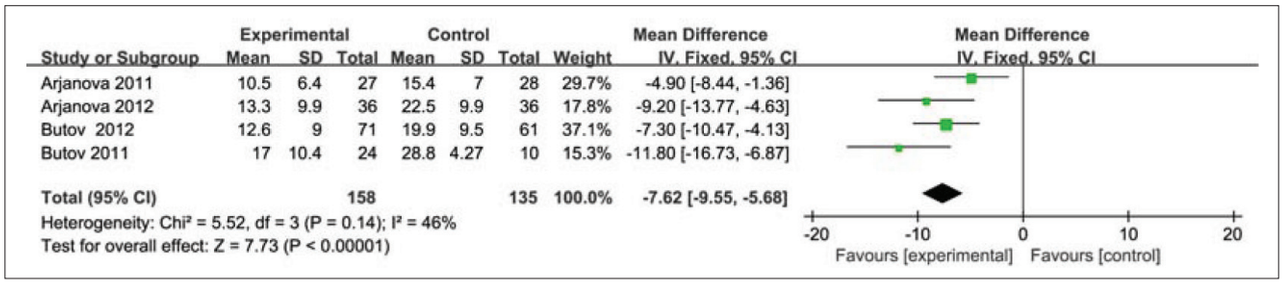

Fig. 4: Meta analysis of erythrocyte sedimentation rate (ESR) in TB patients. Vertical line indicated no difference between the compared two groups (V5 immunitor vs. control). Squares indicated point estimates of mean difference in each individual study, the size of the squares indicated the weight of the corresponding study in the meta-analys, 95%CIs of point estimates were shown by horizontal lines. Pooled mean difference and its 95%CI are shown by diamond shape (like in Figs 6, 8 and 9).

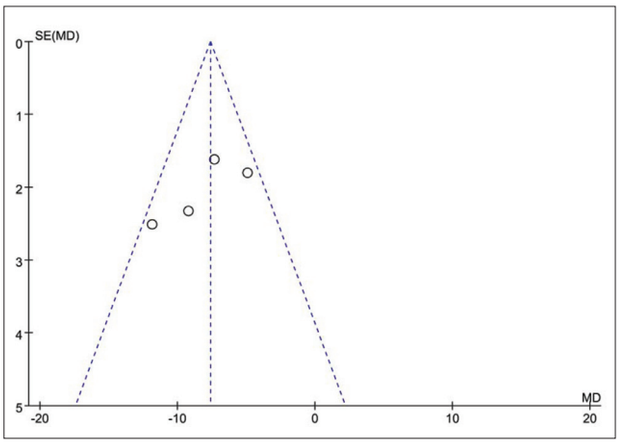

Fig. 5: Funnel plot for the outcome of erythrocyte sedimentation rate (ESR) in TB patients.

mean difference and its 95% CI was 0.96 (-1.13, 3.05), with no statistical significance (P=0.37) (Fig. 8).

2.3.3. *Effect on temperature*

Two clinical trials were included when analyzing the impact on temperature (Arjanova et al. 2012a; Arjanova et al. 2011), the fixed effect model was applied at the end of the follow-up period, the pooled mean difference and its 95% CI was -0.34 (-0.46, -0.22), with statistical significance (P<0.00001) (Fig. 9).

2.3.4. *Adverse events*

None of the included studies reported any systemic adverse events or reactivation of TB (Arjanova et al. 2012; Arjanova et al. 2011; Butov et al. 2012; Butov et al. 2011).

3. Discussion

Immunitor V-5 was derived from the pooled blood of hepatitis B and C carriers. It requires heat and chemical inactivation and

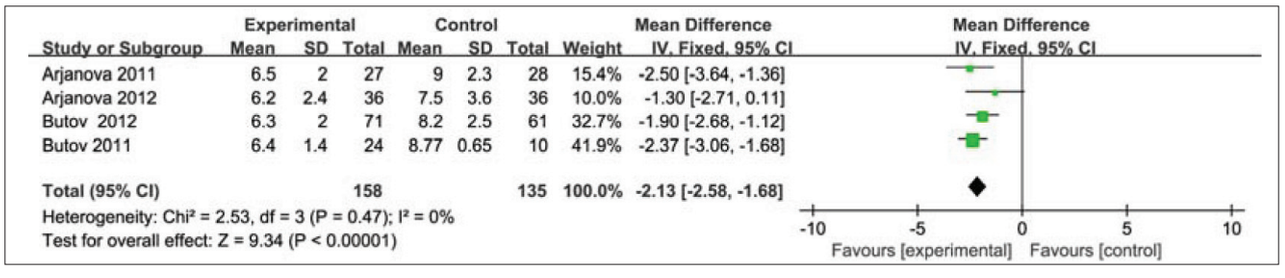

Fig. 6: Meta analysis of leukocyte accounts in TB patients.

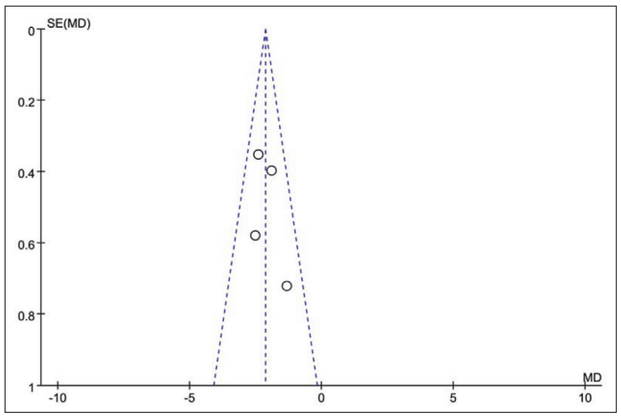

Fig. 7: Funnel plot for the outcome of leukocyte accounts in TB patients.

formulation into a tablet for clinical application. V5 immunitor was approved in 2008 by the Ministry of Health of Ukraine as an immunomodulating supplement for the management of chronic hepatitis. It was currently approved in Ukraine as an immunomodulating supplement, presented as an 850-mg coated pill. The recommended dose was one to two pills per day. The preparation was stable at ambient temperature for 5 years. It was reported that V5 immunitor inherently had circulating *M. tuberculosis* antigens, but the exact content of *M. tuberculosis* antigens was unknown (Arjanova et al. 2011; Batdelger et al. 2009; Yang et al. 2011).

reduced after using V5 immunitor compared to the control group (P<0.00001), indicating that V5 immunitor had am important potential in inhibiting inflammatory reaction in TB patients.

But when analyzing the impact of V5 immunitor on body weight of patients, there was no significant difference between V5 immunitor and the control group (P=0.37). Several factors may account for this: Firstly, the limited subjects were subjected to short follow-up time points with 3 trials for only 1 month and 1 trial for 3 or 3.5 months; Secondly, there were only 4 clinical trials included.

Only two clinical trials focus on the analysis of temperature after using V5 immunitor, and a significant difference was found between V5 immunitor treatment and the control group, implying that V5 immunitor may have some capability to decrease the temperature of TB patients.

The quality assessment showed that in general, these four clinical trials had relatively good quality. But there are several limitations of our study: first, the follow-up period was not long enough to obtain the results of long-term effects, for example, body weight change, recurrence, drug resistance and so on, and the follow-up time points varied across studies. Secondly, the total number of included trials was limited as only four studies met the inclusion criteria. Thirdly, data regarding radiological appearances, cavity closure rates and immunological indicators (e.g. IL-2, IFN-γ and TNF-α) were not available from the studies included. Fourthly, the data like temperatures were just analyzed in two clinical trials.

In conclusion, V5 immunitor was a well-tolerated and helpful addition to treatment for TB patients in terms of significantly improved sputum conversion and reduced inflammation.

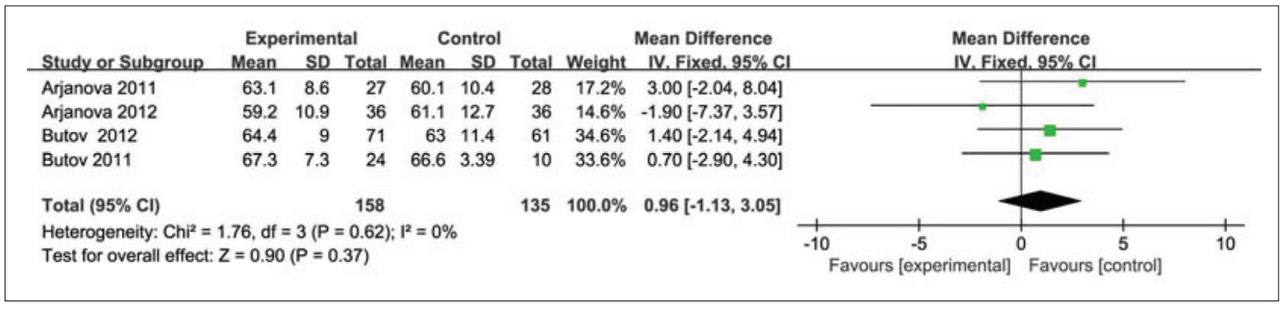

Fig. 8: Meta analysis of body weight of TB patients.

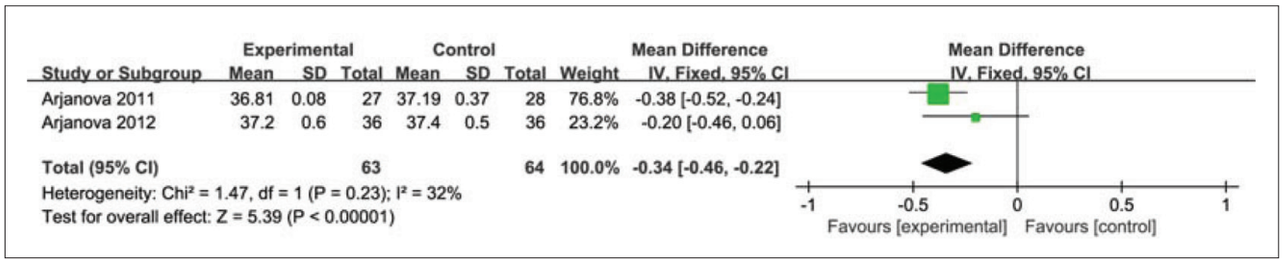

Fig. 9: Meta analysis of temperatures of TB patients.

The meta-analysis of sputum culture conversion rates showed an important statistical significance at the end of following up period between the V5 immunitor group and the control group (P<0.00001), implying that V5 immunitor as adjunctive therapy can substantially help to improve sputum conversion to AFB- in TB patients including drug-sensitive, drug-resistant TB patients or HIV–TB co-infection patients.

As for inflammation biomarkers (i.e. ESR and leukocyte accounts), both of ESR and leukocyte accounts were significantly

4. Experimental

4.1. Study eligibility criteria (PICOS)

Participants (P): TB patients with sputum smear positive for acid-fast bacilli or culture positive for *Mycobacterium tuberculosis*.

Intervention (I): Oral therapeutic vaccine V5 Immunitor as an adjunct to standard, individual or palliative anti-TB treatment.

Control (Comparison) (C): Placebo plus anti-TB treatment, or anti-TB treatment alone.

Outcome (O): Primary outcome: Conversion to sputum smear or culture negativity. Secondary outcomes:

Inflammation: erythrocyte sedimentation rate (ESR) and leukocyte accounts.
Body weight.
Temperature.
Adverse events.
Study design (S): The studies selected for analysis were either randomized controlled trails (RCT) or controlled clinical trials (CCT) without randomization. We did not use these terms as a restriction when searching the database, but filtered the articles by reading the abstract (and when necessary, the full-length article, or by contacting the authors) in order to classify the studies.

4.2. Search strategy

English databases of the Cochrane Controlled Trials Register, MEDLINE, EMBASE, BIOSIS, SCI and Chinese databases of CBM, CNKI, VIP were searched until March 2016 using keywords of V5 Immunitor and tuberculosis, without limitation of language, and the references of eligible studies were also searched. When the full length article was not available from the databases, we contacted the author asking for it.

4.3. Quality assessment in individual studies

Two reviewers independently performed data extraction and quality assessment. Four items were used to assess the quality of included studies based on Cochrane Collaboration recommended criteria: adequate sequence generation, allocation concealment, blinding, and addressing the problem of incomplete outcome data.

4.4. Risk of bias across studies

The selective reporting within studies was assessed by answering whether the results were fully reported as the study was pre-specified.

4.5. Statistical analysis

Statistical analysis was carried out using Revman 5.3 software. The heterogeneity test for the included studies was applied and p values of less than 0.05 were considered as statistical significance. The fixed effect model was applied. $G \pm 1.96 S_G$ (G-Geometric mean, S_G-standard error of geometric mean) was used to describe continuous variables and Risk Ratios (RR) and their 95% confidence interval (95 % CI) were used for binary variable.

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Conflict of interest: None declared.

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