The efficacy of thymosin α1 as immunomodulatory treatment for sepsis: a systematic review of randomized controlled trials

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Abstract

Background: Thymosin α1 (Tα1) as immunomodulatory treatment is supposed to be beneficial for the sepsis patients by regulating T cell subsets and inflammatory mediators. However, limited by the small sample size and the poor study design, the persuasive power of the single clinical studies is weak. This meta-analysis aimed to investigate the impact of Tα1 on the sepsis patients.

Methods: We searched for the Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, CBM, VIP, CNKI, WANFANG, Igaku Chuo Zasshi (ICHUSHI) and Korean literature databases reporting the effects of Tα1 on outcomes in sepsis patients.

Results: Among 444 related articles, 19 randomized controlled trials (RCTs) met our inclusion criteria. Mortality events were reported in 10 RCTs included 530 patients, and the meta-analysis showed significant decrease in Tα1 group compared with control group (RR 0.59, 95% CI 0.45 to 0.77, p = 0.0001). The subgroup analysis showed no difference between the two dosages (RR 0.59, 95% CI 0.43 to 0.81; RR 0.59, 95% CI 0.35 to 0.98, respectively). In 9 RCTs, with a total of 489 patients, Tα1 administered once per day decrease APACHE II score significantly (SMD −0.80, 95% CI −1.14 to −0.47, p < 0.0001) while Tα1 twice per day showed no effect (SMD 0.30, 95% CI 0.10 to 0.70, p = 0.14). However, the length of ICU stay, the incidence of multiple organ failure (MOF) and duration of mechanical ventilation were not significantly affected by Tα1 treatment (SMD −0.52, 95% CI −1.06 to 0.11, p = 0.06; SMD −0.49, 95% CI −1.09 to 0.11, p = 0.11; SMD −0.37, 95% CI −0.90 to 0.17, p = 0.17, respectively). As to the immunological indicators, the level of HLA-DR were increased by Tα1 (SMD 1.23, 95% CI 0.28 to 2.18, p = 0.01) according to the pooled analysis of 8 studies involving 721 patients. Lymphocyte subsets CD3, CD4 and cytokines IL-6, IL-10 and TNF-α were also beneficially affected by Tα1 treatment.

Conclusions: Tα1 may be beneficial to sepsis patients in reducing mortality and modulating inflammation reactions. However, the quality of evidence supporting the effectiveness is low considering the small sample sizes and inadequate adherence to standardized reporting guidelines for RCTs among the included studies.

Keywords: Thymosin α1, Sepsis, Systematic review, Immunomodulatory
Background
Sepsis is a major cause of morbidity and mortality in developed countries [1]. The mechanism of the sepsis syndrome is not completely understood though we do know it includes a systemic immune system response in multiple and complex pathways which is named SIRS (Systemic Inflammatory Reaction Syndrome) [2, 3]. Sepsis begins with epitope shifting from antigen presenting cells into neutrophils, macrophages and T helper lymphocytes (Th), followed by cell transcription factor NF-kB activating, entering nucleus and forming a complex with DNA. Subsequently, apoptosis is induced and Th lymphocytes is activated into Thl cells, which release a large amount of proinflammatory cytokines and chemokines, such as TNF-α, IL-6, IL-1β, IFN-γ and monocyte chemoattractant protein (MCP-1), then complement and coagulation system were activated, and systematic inflammation was developing, leading to high fever, shock, coagulation dysfunction and multiple organ failure, and even death [4–6].
Thymosin α1 (Tα1) is an acidic polypeptide consisting of 28 amino acids extracted and purified from the thymosin fraction 5. Pharmacological studies showed that Tα1 stimulates endogenous IFN-γ secretion, and enhances T cells and the whole immune system [7–12]. Pharmacokinetic studies in healthy volunteers showed good absorption after subcutaneous injection with a peak serum level at between 1 and 2 h and a half-life of less than three hours [13]. Tα1 is approved mainly in countries of Asia and South America, for the treatment of chronic hepatitis B and C as a vaccine enhancer [14]. Although some clinical trials demonstrated that Tα1 is beneficial for the treatment of sepsis by regulating T cell subsets and inflammatory mediators [15–17], the results are less persuasive due to the small sample size and the poor study design. As the influence of Tα1 on prognosis of patients with sepsis remains inconclusive, this systematic review aims to quantitatively evaluate the efficacy and safety of Tα1 in the treatment of sepsis.

Methods
Inclusion criteria
Studies are included if the following criteria are met: 1) Randomized controlled trials (RCTs); 2) Evaluating adult sepsis patients. We defined sepsis according to internationally accepted diagnostic criteria developed on 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference [18]. 3) Comparing Tα1 as add on therapy with no treatment or placebo on the basis of standard or conventional treatment of sepsis in both groups. Standard or conventional treatment is defined as regular treatment for sepsis including adequate empiric antibiotic therapy, ventilation regimen, blood glucose control, resuscitation and hemodynamic support, organ support, sedation or analgesia as needed and adequate nutrition.

Outcome measures
The primary outcomes are death from any cause assessed 28, 60 and 90 days after the initiation of treatment assignment, the length of ventilation and the length of ICU stay. The secondary outcomes included dynamic changes of Sequential Organ Failure Assessment (SOFA), multiple organ dysfunction syndrome (MODS), Acute Physiology and Chronic Health Evaluation II (APACHE II), T lymphocyte subsets, CD4+/CD8+, monocyte human leukocyte antigen-DR (mHLA-DR) expression, and cytokines including IL-6, IL-10 and TNF-α measured on day 0 (the day of enrollment) and 7 in both groups. The rate of adverse drug reactions was taken as indicator for tolerability.

Search strategy
We searched Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library, Issue 3 of 12, March 2016), MEDLINE (January 1966 to April 19, 2016), EMBASE (January 1980 to April 19, 2016) for published studies and Clinicaltrials for registered studies in English [19]. We searched China National Knowledge Infrastructure (CBM), VIP Database for Chinese Technical Periodicals (VIP), Chinese National Knowledge Infrastructure (CNKI) and Wanfang Data in Chinese, all from inception to April 19, 2016. We searched Igaku Chuo Zasshi (ICHUSHI) for Japanese literature, and Korean literature up to February 12, 2015 [20]. We checked the bibliographies in reports of the randomized trials, review articles, and meta-analyses to identify other potentially eligible studies. We used a combination of keywords related to the names of thymosin α1 (Tα1 or Thymosin-alpha (1) or Thymalfasin or Thymalfasine or Thymalsasinum or Timalfasin or Zadaxin) and the type of sepsis-associated disease (“severe infection” or “sepsis” or “septic shock”).

Study selection
Two review authors (FL and HMW) checked titles and abstracts identified from the register, obtained the full text of all potentially relevant studies for independent assessment. The authors decided independently which trials fitted the inclusion criteria and resolved disagreements by discussion or consulting the third author (XZ). The reasons for excluding studies from the review were documented and justified.

Data extraction and management
Two review authors (FL and HMW) performed data extraction independently with a pre-tested electronic table. Discrepancies were resolved by consensus or a third author’s (XZ) adjudication. The following data were abstracted from each study: characteristics of the studies, characteristics of the included patients and outcomes of the studies. The first or corresponding author of each
included study was contacted for clarifications and further information when required.

Assessment of risk of bias in included studies
We used a domain-based evaluation as recommended by the Cochrane Handbook 5.0.2 for Systematic Reviews of Interventions [21]. The following domains were assessed: 1) random sequence generation; 2) allocation concealment; 3) blinding of participants, personnel and outcome assessors; 4) incomplete outcome data; 5) Selective reporting. 6) Bias from other source. We graded these items as having high, low or unclear risk. When discrepancies between review authors existed, we reassessed the studies and reached agreement by consensus.

Statistical analysis
We calculated the treatment effect across trials using the Cochrane statistical package, Review Manager 5.3 (RevMan). We expressed results as risk ratios (RR) with 95% confidence intervals (CIs) for dichotomous outcomes, such as mortality, and mean differences (MDs) and 95% CIs for continuous outcomes, such as the length of ventilation, the scores of the evaluation scales, the counts of lymphocytes subsets and the concentration of cytokines. Heterogeneity among studies was assessed using a Chi² test of heterogeneity (P value < 0.1) and the I² statistic [22]. Trials comparing similar regimens were pooled using fixed effect model, unless significant heterogeneity was observed when using random-effects model. If the mean and SD of the continuous outcomes were not reported in the studies, we assigned the median as the mean if sample size was greater than 25 and estimated the SD from the range (that is, SD range 0.95/4 or interquartile range/1.35) as suggested by Hozo et al. [23]. If sample size was less than 25 we used formulas suggested by Hozo et al. to calculate the mean [23]. If we could not calculate the mean or SD from the available data, we excluded the study from the analysis.

Sensitivity analysis
We undertook sensitivity analyses taking into account the quality of the studies. To evaluate a single study’s effect on the pooled data, sensitivity analysis was carried out by excluding each study. Publication bias was evaluated using Funnel plots and Fail-Safe Number (Nfs) [24].

Subgroup analysis and investigation of heterogeneity
We explored sources of heterogeneity with a priori subgroup hypotheses: dosage regimen of Ta1. Patients received subcutaneous injections of 1.6 mg Ta1 (ZADAXIN™, SciClone Pharmaceuticals, Foster City, CA, USA) twice per day for 5 consecutive days, then once per day for 2 consecutive days.
| Study       | Population                                                                 | Case number, Tα/ control | Interventions in Tα group                  | Interventions in control group | Outcomes                                                                 |
|------------|-----------------------------------------------------------------------------|---------------------------|------------------------------------------|---------------------------|---------------------------------------------------------------------------|
| Chen XL 2009 [25] | Sepsis patients in ICU, age over 18 years                                    | 40, 20/20                 | SSC therapy + Tα1, 1.6 mg, SC, QD        | SSC therapy + NS          | Levels of CD3, CD4, CD8, CD4/CD8, NK, CRP, APACHE II                   |
| Cheng AB 2010 [26] | Sepsis patients in ICU, age under 70 years and HLA-DR < 30 %                | 60,30/30                  | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment + NS | Levels of CD4, CD8 and HLA-DR                                           |
| Gui CM 2012 [27] | Sepsis patients in ICU, age between 18 and 80 years                         | 42,22/20                  | SSC therapy + Tα1, 1.6 mg, SC, QD        | SSC therapy               | Levels of CD4, CD4/CD8, igg, igm, PCT, IL-6, IL-10, and APACHE II        |
| Hu XL 2007 [28] | Abdominal sepsis patients in ICU                                            | 45,24/21                  | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment + NS | Levels of TNF-α, IL-6, IL-10, CD3, CD4, CD8, CD4/CD8, NK and 28-day mortality |
| Gong ZH 2011 [29] | Sepsis patients in ICU, age under 70 years                                  | 56,28/28                  | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment   | Levels of TNF-α, and WBC                                                 |
| Chen J 2007 [38] | Septic shock, APACHE II scores between 15 and 20                           | 42,21/21                  | SSC therapy + Tα1, 1.6 mg, SC, BID       | SSC therapy               | Levels of T-lymphocyte subtype, natural killer cell and mechanical ventilation time, length of ICU stay, 28-day mortality |
| Fan JB 2014 [30] | Sepsis patients or septic shock                                             | 120,60/60                 | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment   | Levels of CD4, CD8, CD4/CD8, APACHE II and 28-day mortality             |
| Lei S 2005 [37] | Severe hospital acquired pneumonia patients in ICU, HLA-DR < 30 %           | 38,21/17                  | Conventional treatment + Tα1, 1.6 mg, SC, BID | Conventional treatment   | Levels of CD4, CD8, CD4/CD8, NK, HLA-DR and 28-day mortality           |
| Li YN 2009 [31] | Age over 18 years, suffering from severe sepsis with Marshall score over 5 | 47, 23/24                 | SSC therapy + Tα1, 1.6 mg, SC, QD        | SSC therapy               | Levels of HLA-DR, CD3, CD4, CD8, length of ICU stay, 28-day mortality    |
| Wu JN 2004 [32] | Sepsis patients in ICU, HLA-DR < 30 %                                       | 44,22/22                  | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment + NS | Levels of HLA-DR, CRP, APACHE II; and MOF                               |
| Wu JF 2013 [39] | Patients in ICU with severe sepsis                                          | 361,181/180               | Conventional treatment + Tα1, 1.6 mg, SC, QD, twice per day for 5 consecutive days, then once per day for 2 consecutive days | Conventional treatment + NS | Levels of HLA-DR, CD4/CD8, WBC, duration of ICU stay, mechanical ventilation time, APACHE II and 28-day mortality |
| Wu JF 2014 [40] | Sepsis patients, age over 18 years                                          | 54,26/28                  | Conventional treatment + Tα1, 1.6 mg, SC, twice per day for 5 consecutive days, then once per day for 2 consecutive days | Conventional treatment   | 28-day mortality                                                         |
| Zhang BJ 2014 [41] | Sepsis patients                                                             | 60,30/30                  | Conventional treatment + Tα1, 1.6 mg, SC, twice per day | Conventional treatment   | Level of IL-6 and APACHE II                                              |
| Zhang Z 2006 [33] | Sepsis patients                                                             | 38,19/19                  | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment   | Levels of CRP, CD3, CD4, CD8, CD4/CD8, NK, and APACHE II              |
| Zhou LX 2009 [35] | Sepsis patients aged > 18, Marshall score > 5                              | 47, 23/24                 | Tα1 plus SSC therapy                     | SSC therapy               | IL-6, IL-10, TNF-α, HLA-DR, T lymphocytes, 28-day mortality            |
| Zhao MY 2007 [34] | Sepsis patients in ICU, HLA-DR < 30 %, age < 70                             | 42,21/21                  | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment + NS | Levels of HLA-DR, CD4, CD8, TNF-α, IL-6 and IL-10                        |
| Study          | Disease Description | Age | Treatment 1 | Treatment 2 | Outcome Measures |
|---------------|---------------------|-----|-------------|-------------|-----------------|
| Zhou Q 2011 [36] | Severe sepsis, age > 18 years | 82,42/40 | SSC therapy + Tα1, 1.6 mg, SC, QD | SSC therapy | Levels of HLA-DR, CD3, CD4, CD8, CD4/CD8 |
| Zhu 2015 [43] | Severe sepsis, age > 18 years | 60,30/30 | Conventional treatment + Tα1, 1.6 mg, SC, QD | Conventional treatment + NS | Levels of, CD3, CD4, CD8, CD4/CD8, duration of ICU stay and APACHE II |
| Lu 2015 [42] | Patients with sepsis, age > 18 years | 76,38/38 | Conventional treatment + Tα1, 1.6 mg, SC, twice a week | Conventional treatment + NS | Levels of, CD3, CD4, CD8, CD4/CD8 |
Primary analysis of 28-day mortality
A total of 10 studies reported mortality within 28 days, including a total of 530 patients and 158 events (Fig. 2). No significant heterogeneity was found across the 10 studies (Chi $3.81$, $I^2$ $0\%$, $p = 0.92$). Furthermore, we detected no evidence of publication bias after a funnel plot analysis (Fig. 3), and $N_{f_{0.05}} = 36.04$. The RR showed a significant decrease of mortality in $\text{Ta}1$ group compared with control group (RR $0.59$, 95 \% CI $0.45$ to $0.77$, $p = 0.0001$) (Fig. 2).

Subgroup analysis of 28-day mortality
To explore the relationship between different dose of $\text{Ta}1$ and 28-day mortality, we conducted the subgroup analyses. The intervention of $\text{Ta}1$ administered once per day was adopted in 7 trials involving 396 patients and the intervention of $\text{Ta}1$ administered twice per day was adopted in three trials involving 134 patients. The subgroup analysis showed both the two dosage regimens significantly decreased mortality of sepsis patients ($\text{Ta}1$ once per day: RR $0.59$, 95 \% CI $0.43$ to $0.81$; $\text{Ta}1$ twice per day: RR $0.59$, 95 \% CI $0.35$ to $0.98$) (Fig. 2).

The impact on APACHE II
Primary analysis
Nine studies involving 489 patients reported APACHE II score. There was a significant difference in APACHE II score reduction between $\text{Ta}1$ and control group (SMD $-0.55$, 95 \% CI $-0.97$ to $-0.13$, $p = 0.01$), which meant that $\text{Ta}1$ decreased APACHE II in a greater degree than control group. Since the heterogeneity was high (Chi $39.82$, $I^2$ $80\%$) (Fig. 4) among different studies, we conducted subgroup analysis.

Subgroup analysis
The intervention of $\text{Ta}1$ administered once per day was adopted in 7 trials involving 391 patients and the intervention of $\text{Ta}1$ twice per day was adopted in two trials involving 98 patients (Fig. 4). SMD for $\text{Ta}1$ once per day was $-0.80$ (95 \% CI $-1.14$ to $-0.47$, $p < 0.00001$) with a moderate heterogeneity (Chi $14.26$, $I^2$ $58\%$, $p = 0.03$). However, the effect of $\text{Ta}1$ twice per day on APACHE II was not statistically significant (SMD $0.30$, 95 \% CI $-0.10$ to $0.70$, $p = 0.14$). No significant heterogeneity was found across the 2 studies (Chi $0.34$, $I^2$ $0\%$, $p = 0.56$).

The impact on MOF
Only one study involving 44 patients reported the incidence of multiple organ failure (MOF). As shown in Table 3, there was no significant difference on MOF between $\text{Ta}1$ and control group. (SMD $-0.49$, 95 \% CI $-1.09$ to $0.11$, $p = 0.11$).

| Table 2 Risk of bias in include studies |
|---------------------------------------|
| The first author | Publication year | Adequate sequence generation | Allocation concealment | Blinding (Participant) | Blinding (Personnel) | Blinding (Outcome assessor) | Incomplete outcome data (attrition bias) | Selective reporting (reporting bias) | Other bias |
|------------------|-----------------|-----------------------------|------------------------|-----------------------|---------------------|---------------------------|-------------------------------|-------------------------------|------------|
| Chen XL          | 2009            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Cheng AB         | 2010            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Gui CM           | 2012            | High risk                   | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Hu XY            | 2007            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Gong ZH          | 2011            | Low risk                    | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Chen J           | 2007            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Fan JB           | 2014            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Lei S            | 2005            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Li YN            | 2009            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Wu JN            | 2004            | Low risk                    | Low risk               | Low risk              | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Wu JF            | 2013            | Low risk                    | Low risk               | Low risk              | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Wu JF            | 2014            | Low risk                    | Low risk               | Low risk              | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Zhang BJ         | 2014            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Zhang Z          | 2006            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Zhou LX          | 2009            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Zhao MY          | 2007            | Low risk                    | Low risk               | Low risk              | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Zhou Q           | 2011            | Low risk                    | Low risk               | Low risk              | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Zhu SJ           | 2015            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
| Lu FP            | 2015            | Unclear                     | Unclear                | Unclear               | Unclear             | Low risk                  | Low risk                      | Low risk                      | Low risk   |
The impact on mechanical ventilation days
Six studies reported duration of mechanical ventilation, and a total of 570 patients were included. As shown in Table 3, there was no significant difference on the mechanical ventilation days between Tα1 and control group (SMD −0.37, 95 % CI −0.90 to 0.17, p = 0.17). However, heterogeneity was high (Chi 32.24, I² 84 %, p < 0.001).

The impact on length of ICU stay
Six studies involving 591 patients reported the length of ICU stay. As shown in Table 3, there was no significant difference on the length of ICU stay between Tα1 and control group. SMD was −0.52 (95 % CI −1.06 to 0.01, p = 0.06) with high heterogeneity (Chi 34.92, I² 86 %, p < 0.0001).

The impact on HLA-DR levels
Eight studies including 721 patients reported the level of HLA-DR. There was a significant difference in HLA-DR between Tα1 and control group. SMD was 1.23 (95 % CI 0.28 to 2.18, p = 0.01), with a high heterogeneity (Chi 179.65, I² 96 %) (Fig. 4). To explore the high heterogeneity among different studies, we conducted subgroup analysis.
**Subgroup analysis**

The group of Tα1 administered once per day included six trials with 322 patients and the group of Tα1 administered twice per day included two trials with 399 patients. The subgroup analysis showed a significant effect of Tα1 once per day on HLA-DR (SMD 0.86, 95 % CI 0.50 to 1.23, \( p < 0.001 \)) with a moderate heterogeneity (Chi 12.24, I² 59 %, \( p = 0.03 \)). However, the effect of Tα1 twice per day on HLA-DR was not statistically significant (SMD 2.26, 95 % CI -0.12 to 4.64, \( p = 0.06 \)) with a significant heterogeneity (Chi 39.47, I² 97 %, \( p < 0.001 \)) (Fig. 5).

The impact on T lymphocyte subsets

Tα1 showed significantly better effect on CD3⁺, CD4⁺ and CD4⁺/CD8⁺ than control, but didn’t show difference on CD8⁺. The pooled results were showed in Table 4. However, there was a high heterogeneity across these studies.

**The impact on cytokines**

**IL-6 levels**

Four studies involving 189 patients reported the level of IL-6. There was no significant difference on the level of IL-6 between Tα1 and control group (SMD -0.32, 95 % CI -1.24 to 0.60, \( p = 0.49 \)), and the heterogeneity across the four studies was high (Chi 28, I² 89 %, \( p < 0.0001 \)) (Table 5).

**IL-10 levels**

Three studies involving 129 patients reported the level of IL-10. There was a significant difference on the level of IL-10 between Tα1 and control group (SMD 1.06, 95 % CI 0.64 to 1.49, \( p < 0.0001 \)). No significant heterogeneity was found across the 3 studies (Chi 2.63, I² 24 %, \( p = 0.27 \)) (Table 5).

**TNF-α levels**

Four studies involving 190 patients reported the level of TNF-α. There was a significant difference on the level of TNF-α between Tα1 and control group (SMD -0.47, 95 % CI -0.76 to -0.18, \( p = 0.002 \)). No significant heterogeneity was found across the 5 studies (Chi 2.55, I² 0 %, \( p = 0.47 \)) (Table 5).

**Safety of Tα1**

The included RCTs reported neither Tα1 related severe adverse event nor treatment discontinuation due to intolerance or adverse events of Tα1.

**Discussion**

In this systematic review of RCTs including 1354 patients with sepsis, we found benefits of Tα1 on both survival and other clinical indicators. We also explored the efficacy of Tα1 on immune parameters.

**Overall completeness and applicability of evidence**

The trials were identified following a systematic search of the literature in multi-language databases. Besides English and Chinese databases, we additionally searched...
Japanese and Korean database to enhance our systematic review's ability of reflecting international practice. Study inclusion criteria were tightly defined and the meta-analysis was rigorously conducted according to a pre-defined analysis plan addressing specific hypotheses. We didn't set limitations on the primary etiologies of sepsis, however, all trials included critically ill patients where a common systemic inflammatory pathway was activated. Therefore, we think that there is a good biologic reason to perform a broad meta-analysis, which also considerably increases the generalizability and usefulness of the review.

The data of lymphocyte subsets and cytokines were collected at the 7th day of treatment course of Tα1 from the included studies and had the same tendency of favoring Tα1 group as 28 days’ mortality, which indicates that those data can be served as prognosis indicator for sepsis. Our systematic review is consistent with recent studies indicated that the relationship of cytokines and mortality of sepsis [44–46].

In subgroup analysis, both Tα1 1.6 mg once daily and 1.6 mg twice daily decreases mortality, APPACHE II score, ventilation days and ICU days, and they also showed positive effect on lymphocyte subsets and cytokines. Though we didn't carried out comparisons between the two regimens, we recommend Tα1 1.6 mg once daily to be used for cost-effectiveness considerations.

The role of Tα1 in immune modulatory therapy of sepsis
It was indicated in a variety of studies that Tα1 modulated immune functions through multiple pathways; however, its mechanism was not fully established [47]. Recent studies suggested that Tα1 combines to toll like receptors (TLRs) located in the surface of dendritic cells (DC), and thus activates them into effector cells with the function of stimulating or inhibiting T cells [48]. As highly specialized type of antigen-presenting cell (APC), DC activate CD3⁺ (total T cells), CD4⁺ (helper T cells), and CD8⁺ (cytotoxic T cells), which is considered as important pathway for Tα1 to reverse immune suppression in sepsis. Moreover, plasmacytoid dendritic cells promote the function of regulatory T Cells, which increase the production of anti-inflammatory factors, such as IL-10 and TGF-β, and reduce the pro-inflammatory cytokines, such as IL-2, IL-6 and TNF-α, so that to combat against the pro-inflammatory cytokines storm in early period of sepsis and then modulate the over-stimulating of nonspecific immunity in the deferment period later on [49].

HLA-DR is expressed in the surface of B-lymphocytes, macrophages, activated T lymphocytes and other immune cells, and the decline of HLA-DR expression is proposed as a reflection of immunosuppression in critically ill patients [50].

Our study shows Tα1 increased CD3⁺, CD4⁺, and CD4⁺/CD8⁺, as well as the level of HLA-DR, which

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**Table 4** The influence on lymphocyte subsets

| Lymphocyte subsets | Included studies | Cases | Chi | I² % | SMD | 95 % CI | P |
|--------------------|------------------|-------|-----|------|-----|--------|---|
| CD3                | 9 [25, 28, 31, 33, 35, 36, 38, 42, 43] | 521   | 56.12 | 86 % | 0.84 | 0.35, 1.33 | 0.0008 |
| CD4                | 14 [25–28, 30, 31, 33–38, 42, 43]    | 779   | 51.01 | 75 % | 0.80 | 0.50, 1.10 | <0.0001 |
| CD8                | 12 [25, 26, 28, 30, 31, 33–37, 42, 43] | 695   | 62.64 | 82 % | −0.27 | −0.64, 0.10 | 0.16 |
| CD4⁺/CD8           | 13 [25, 27, 28, 30, 31, 33, 35–39, 42, 43] | 1038  | 102.37 | 88 % | 0.62 | 0.22, 1.02 | <0.0001 |

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**Fig. 5** The effect of thymosin α1 on HLA-DR levels. Eight studies including 721 patients reported the level of HLA-DR. There was a significant difference in HLA-DR between thymosin α1 and control group.
comply with the results of basic research. However, Tα1 did not demonstrate a significant impact on CD8+. As to the influence on cytokines, our study showed that Tα1 decreased the level of TNF-α and increased IL-10, but had no significant effect on IL-6. Besides that, it was suggested by both basic research and clinical trials that multiple kinds of other cytokines experience significant changes with the progress of sepsis, including IL-2, IL-3, IL-4 and IFN-γ etc. However, those cytokines were barely evaluated in the included studies. Therefore, more studies, both fundamental and clinical are needed for further understanding the immune-modulatory effect of Tα1 on different immune cells and cytokines with the progress of sepsis.

**Safety of Tα1**

According to package insert of Tα1, the rate of adverse reactions of Tα1 is less than 1 % across all its indications. The reported ADRs include pain, redness, and transient muscle atrophy in injection site, multiple joint pains with swelling, and rash. Both Li [51] and our systematic reviews included adverse reactions to evaluate Tα1’s tolerability, and no severe ADRs were recorded in the included clinical trials.

**Comparisons with published review**

Three similar systematic reviews had been published recently [51–53]. All of them took Tα1-based immune modulatory therapy as intervention, which means they included not only Tα1, but also concomitantly used ulinastatin. Han [52] evaluated combination of ulinastatin and Tα1, but not Tα1 monotherapy. For Tα1 monotherapy groups, the recently published reviews included fewer studies than ours, which may because they searched less Chinese literature database. For outcomes, all the systematic reviews took mortality as primary outcomes, while durations of mechanical ventilation and ICU stay were evaluated in both Han and Feng’s review, and APACHE II in Feng’s review. Compared to that, our study also included MOF score, hoping to provide some hint on efficacy of Tα1 in organ dysfunction in sepsis though we didn’t come to a definite conclusion because of the limited number of included study. Furthermore, the studies by Li and Feng [51, 53] failed to include immune indicators, while Han evaluated TNF-α and IL-6 [52]. We considered more immune indicators since it was implicated in previous studies that Tα1 showed pleiotropic effects on immune system [54]. We hope the meta-analysis of immune parameters will be helpful to find out the factors to predict the efficacy of Tα1 in sepsis. Furthermore, providing communitive clinical evidence as meta-analysis may indicate directions for subsequent fundamental researches on the immunomodulatory mechanism of Tα1 in treating sepsis.

We didn’t include studies evaluating efficacy of concurrent use of Tα1 and ulinastatin, except for the studies where ulinastatin were used as background therapy. According to present study, it seems that immune-modulatory effects between Tα1 and ulinastatin overlap each other especially when it comes to their effects on proinflammatory mediators. To figure out to what extent Tα1 is responsible for the beneficial effects noted in the clinical trials, we decided to focus on the studies evaluating the efficacy of Tα1 monotherapy. Future studies providing head-to-head comparison between Tα1 and ulinastatin may be beneficial to further discover the pathway by which immune modulators pose influence on physiopathology of sepsis.

**Limitations of the review**

The meta-analysis combined data from a group of predominantly underpowered single center studies. Although there was minimal heterogeneity among trial results on mortality, we are aware that we pooled clinical trials with high risk of bias, thus, the validity of our meta-analysis may be criticized. Another concern is that great heterogeneity existed in the meta-analysis of lymphocyte subsets and cytokines, which may be related to the different measure methods across the included studies. We used standardized mean deviation and carried out sensitivity analysis, and it was showed that the pooled results were stable after removal the studies with heterogeneity.

In 2015, the Society of Critical Care Medicine proposed the new definition of sepsis, which demonstrated sepsis as a life-threatening organ dysfunction (OD) due to a dysregulated host response to infection. SOFA was the major tool to evaluate organ dysfunction, and was shown to be associated with prognosis of sepsis [55]. We included SOFA in our secondary outcomes, regrettfully, none of the included studies used SOFA to evaluate efficacy of Tα1.

**Conclusion**

In summary, Tα1 may have some benefits in reducing 28 day mortality, deceasing APPACHE II score and
modulating immune parameters in sepsis patients, however, the quality of evidence is low. More high-quality studies are needed to confirm Tα1 efficacy in improving clinical outcomes and provide comprehensive understanding of its immunomodulatory role in sepsis.

Key messages
- In sepsis patients, Tα1 decreased 28 days mortality on the basis of regular therapy.
- Both Tα1 1.6 mg once daily and 1.6 mg twice daily had the effect to decrease mortality and APPACHE II score.
- Tα1 increased the level of IL-10 among sepsis patients.
- Tα1 reduced the level of TNF-α among sepsis patients.
- Sepsis patients benefited from Tα1 as immunomodulatory treatment.

Abbreviations
IL: Interleukin; 95 % CIs: 95 % confidence intervals; APACHE II: Acute physiology and chronic health evaluation II; mHLA-DR: Monocyte human leukocyte antigen-DR; MODS: Multiple organ dysfunction syndrome; SR: Systematic review; TNF: Tumor necrosis factor; Tα1: Thymosin α1

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Availability of data and materials
All the data supporting our findings is contained within the manuscript.

Authors’ contributions
Study design: XZ and FL. Data analysis: HMW, FL and TSW. Manuscript writer: HMW, FL, TSW and XZ. All our authors have read and approved of the final version of our manuscript.

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Competing interests
The authors declare that they have no competing interests.

Consent for publication
Our manuscript doesn’t contain any individual person’s data, so it’s not applicable in this section.

Ethics approval and consent to participate
Our manuscript is a secondary-research, which doesn’t contain any individual patient data, so that no consent to participate and ethics approval is needed in theory. We failed to find national guidelines which contains special statement for the ethics of systematic review. We consulted the ethics committee of Peking University Third Hospital, and were informed that no formal ethics approval was required for this research.

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