EVIDENCE-BASED ADVANCE AND MANAGEMENT OF DRUG TREATMENT OF INFECTIOUS DISEASES

EDITED BY: Yonggang Zhang, Hong Fan and Min Yang
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EVIDENCE-BASED ADVANCE AND MANAGEMENT OF DRUG TREATMENT OF INFECTIOUS DISEASES

Topic Editors:
Yonggang Zhang, Sichuan University, China
Hong Fan, Sichuan University, China
Min Yang, Anhui Medical University, China

Infectious diseases are caused by microorganisms that release toxins or invade body tissues. The most common pathogenic organisms are bacteria, viruses, fungi, protozoa, and helminths. Systemic infections usually cause fevers, chills, sweats, malaise, and occasionally headache, muscle and joint pain, or changes in mental status, and even septic shock-MODS. Infectious diseases have always threatened populations and caused great loss of life in history, but since the last century, with the discovery of antibiotics, historical trends have been reversed. It is reported that between 1990 and 2017, age-standardized disability-adjusted life year (DALY) rates decreased by 41.3% (38.8-43.5) for infectious diseases. However, in 2017, lower respiratory infections are still ranked as the third cause of DALYs.

Drug therapy is the one of most critical management strategies for infectious diseases. Efficacy and safety of drug therapy should always be considered, especially in elders, neonates and immunosuppressed patients. Off-label drug therapy has been performed for complex infectious diseases, especially for new emerging infectious diseases. However, off-label drug therapy can be extremely complex: some are prone to rational use, while others are more susceptible to the issues of irrational use. With the increasing rate of publication of data in this area, new evidence for the efficacy and safety of different treatment approaches is constantly developing. Thus, rigorous analysis of such data is imperative, which will guide future clinical practice and guidelines.

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Respiratory Fluoroquinolones Monotherapy vs. β-Lactams With or Without Macrolides for Hospitalized Community-Acquired Pneumonia Patients: A Meta-Analysis

*Sitong Liu[1], Xiang Tong[1], Yao Ma[2], Dongguang Wang[1], Jizhen Huang[1], Li Zhang[1], Man Wu[1], Lei Wang[1], Tao Liu[1] and Hong Fan[1]**

[1] Department of Respiratory Medicine and Critical Care Medicine, West China Hospital/West China School of Medicine, Sichuan University, Chengdu, China, [2] The Center of Gerontology and Geriatrics, West China Hospital/West China School of Medicine, Sichuan University, Chengdu, Sichuan, China

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***Correspondence:**
Hong Fan
fanhongfan@qq.com

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Background: The choice of empirical antibiotic treatment for patients with community-acquired pneumonia (CAP) who are admitted to non-intensive care unit (ICU) hospital wards is complicated by the limited availability of evidence. We systematically reviewed the efficacy and safety of strategies of empirical treatment with respiratory fluoroquinolone monotherapy and β-lactam with or without macrolide for non-ICU hospitalized CAP patients.

Methods: We searched databases including PubMed, the Cochrane Library (Issue11, 2018), EMbase, China National Knowledge Internet (CNKI), WanFang Data, VIP, and China Biology Medicine disc (CBMdisc) to identify randomized controlled trials (RCTs) involving the comparison of respiratory fluoroquinolone monotherapy and β-lactam with or without macrolide for the non-ICU hospitalized patients with CAP up to November 2018. Two reviewers independently screened literature according to the inclusion and exclusion criteria, extracted data, and assessed the risk of bias of the included studies. A meta-analysis was performed with the outcomes.

Results: A total of 22 studies involving 6,235 patients were included. The results of the meta-analysis showed a non-significant trend toward an advantage to the respiratory fluoroquinolone in overall mortality (RR 0.82, 95% CI 0.65–1.02). No significant difference was found between the two strategies in clinical success (the intention-to-treat population: RR 1.03, 95% CI 0.99–1.08; the clinically evaluable population: RR 1.03, 95% CI 0.999–1.055; the population in which it was unclear whether intention-to-treat or per-protocol analysis was used: RR 1.04, 95% CI 0.99–1.09), microbiological treatment success (RR 1.04, 95% CI 0.997–1.092), and length of stay (SMD −0.06, 95% CI −0.16 to 0.04). The advantage of respiratory fluoroquinolone was statistically significant on the drug-related adverse events (RR 0.87, 95% CI 0.77–0.97).

Conclusions: Current evidence shows that fluoroquinolone monotherapy has similar efficacy and favorable safety compared with β-lactam with or without macrolide for non-ICU hospitalized CAP patients. Since the limitation of region, quantity and quality of included studies, more RCTs with large scale and high quality are needed to verify the above conclusion.

Keywords: community-acquired pneumonia, fluoroquinolones, β-lactams, macrolides, systematic review, meta-analysis, randomized controlled trial

INTRODUCTION

Long recognized as a major cause of death, community-acquired pneumonia (CAP) has been studied intensively since the late 1800s (Musher and Thorner, 2014). Despite the development of antimicrobial agents, pneumonia remains a major cause of hospitalization and death worldwide (Thomas et al., 2012; Welte et al., 2012).

Physicians must choose an optimal therapeutic regimen that eliminates the infection effectively, minimizes the risk of developing drug resistance and does not compromise the safety of the patient. Guidelines were written to develop a uniform set of recommendations that would provide appropriate antimicrobial therapy for the majority of patients with CAP. For patients with CAP who are admitted to a non-intensive-care-unit (ICU) ward, most guidelines recommend either respiratory fluoroquinolone monotherapy or β-lactam with or without macrolide for empirical treatment (Mandell et al., 2007; Lim et al., 2009; Woodhead et al., 2011; Cao et al., 2018). In America, guidelines recommend a respiratory fluoroquinolone monotherapy or a β-lactam plus a macrolide for the non-ICU inpatients (Mandell et al., 2007). In Britain, the British Thoracic Society suggests that amoxicillin is preferred for adults hospitalized with low severity CAP, while amoxicillin plus a macrolide is preferred for patients hospitalized with moderate severity CAP (levofloxacin, moxifloxacin, or doxycyline is alternative agent for those intolerant of penicillins or macrolides) (Lim et al., 2009). In Europe, guidelines recommend a respiratory fluoroquinolone monotherapy (levofloxacin or moxifloxacin), or a non-antipseudomonal cephalosporin, or a β-lactam (e.g., aminopenicillin) with or without a macrolide for non-ICU hospitalized patients (Woodhead et al., 2011). In China, a β-lactam (e.g., penicillins-β-lactamase-inhibitor combinations) with or without a macrolide, or respiratory fluoroquinolone monotherapy is suggested for the non-ICU inpatients (Cao et al., 2018). However, there is no consensus on which strategy is the best one. Level-one evidence for the comprehensive comparison of the two strategies is limited.

As main classes of antibiotics that have dominated the market for years, β-lactams, macrolides and fluoroquinolones are active against the major causative agents of CAP with different mechanisms (Walsh, 2003; Raja et al., 2004; Suda et al., 2018). β-lactam antibiotics work by inhibiting cell wall biosynthesis (inhibiting the β-lactam "binding protein" enzymes) in the bacterial organism (Fisher et al., 2005). They are effective against major causative bacteria of CAP (e.g., Streptococcus pneumonia) but not effective against Mycoplasma Pneumoniae (MP) or Chlamydia Pneumoniae (CP). Macrolides inhibit protein biosynthesis by binding to the P site on the 50S subunit of the bacterial ribosome and they are effective against Legionella Pneumophila, mycoplasma and chlamydia (Tenson et al., 2003). Physicians usually prescribe β-lactam plus macrolide for patients with CAP when infection with MP or CP is suspected. Fluoroquinolones eradicate bacteria by inhibiting the replication and transcription of bacterial DNA (preventing bacterial DNA from unwinding and duplicating) (Hooper, 2001; Aldred et al., 2014). Fluoroquinolones, especially respiratory fluoroquinolones (moxifloxacin, gemifloxacin, and levofloxacin) act against the major causative agents of CAP (including major causative bacteria, MP, CP and Legionella Pneumophila) and they are widely used as a monotherapy for patients with CAP.

Researchers from different countries and areas have performed randomized controlled trials (RCTs) to compare the efficacy of the two strategies. However, the results were not consistent. Finch et al. found that monotherapy with moxifloxacin was superior to that with a standard combination regimen of a β-lactam with or without a macrolide in the treatment of patients with CAP admitted to a hospital (Finch et al., 2002). Similarly, Huang G et al. reported that moxifloxacin was superior to cefuroxime with azithromycin in inpatients with low-moderate severity CAP (Huang et al., 2008). On the contrary, Erard et al. found that there were no significant differences between levofloxacin monotherapy and ceftriaxone with or without clarithromycin in non-ICU hospitalized CAP patients (Erard et al., 2004). Li BH et al. also reported that no significant differences were found between levofloxacin and cefuroxime with azithromycin in non-ICU hospitalized CAP patients (Li et al., 2009). Additionally, the small amount of patients enrolled in each trial limited the validity of the results.

Therefore, we conducted a systematic review and meta-analysis to conclusively and comprehensively compare the efficacy and safety of respiratory fluoroquinolone monotherapy vs. β-lactam with or without macrolide for empirical treatment for non-ICU hospitalized CAP patients.

METHODS

Search Strategy

We searched databases including PubMed, the Cochrane Library (Issue11, 2018), EMbase, CNKI, WanFang Data, VIP and China Biology Medicine disc (CBMdisc) to identify RCTs

up to November 2018. Search terms were "community-acquired pneumonia," "fluoroquinolones" or "levofloxacin" or "moxifloxacin" or "gemifloxacin," and "macrolides" or "β-lactams." The search was restricted to RCTs. The language of the research papers was restricted to English and Chinese. All reference lists from relevant articles and reviews were hand-searched for additional eligible studies. We did not include abstracts from conferences because there is frequently considerable difference between data presented in conference abstracts and the subsequent peer-reviewed publications.

Study Selection

Two reviewers (SL and XT) independently carried out the literature search and examined relevant RCTs for further assessment. A checklist was used to assess whether studies met our inclusion criteria: (1) population: hospitalized patients diagnosed with CAP; (2) exposure: one of levofloxacin, moxifloxacin or gemifloxacin; (3) comparison group: β-lactams with or without macrolides; (4) outcome: at least include one of mortality, clinical treatment success, microbiological treatment success, length of hospital stay or adverse events; (5) study design: RCTs. Exclusion criteria eliminated duplicate reports and studies on patients aged <18 years, outpatients, critically ill patients admitted to ICU, or patients identified as having some form of healthcare-associated pneumonia (HCAP).

Data Extraction

Two reviewers (SL and XT) independently extracted data from the trials included in the meta-analysis using a predesigned review form. In case of any disagreement between the two reviewers, a third reviewer extracted the data and the results were attained by consensus. The authors of trials were contacted for missing data when necessary. Data on first author, publication details, study design, included population, drug tested, endpoint data and adverse events during the treatment were extracted.

Assessment of Risk of Bias

Two reviewers (SL and XT) independently assess the risk of bias of the RCTs included in the meta-analysis. We use the domain-based method as recommended in The Cochrane Hand-book (Higgins and Altman, 2011a) according to: sequence generation, allocation concealment, blinding, incomplete outcome data addressed, free of selective reporting, and free of other bias. A third review author was responsible for resolving disagreements.

Outcomes

The primary outcome was all-cause mortality during the study period (treatment and follow-up period). Secondary outcomes included: clinical treatment success ("cure" was defined as resolution of all symptoms and signs of infections; "improvement" was defined as resolution of two or more of the baseline symptoms or signs of infections) (Frank et al., 2002; Writing Group of Guidance for Clinical Trials of Anti-bacterial Drugs, 2014) assessed at the test-of-cure (TOC) visit in the intention-to-treat population and clinically evaluable population; microbiological treatment success (defined as the eradication of baseline pathogens, or as presumed eradication based on the clinical outcomes when post-treatment cultures were not performed) (Frank et al., 2002; Writing Group of Guidance for Clinical Trials of Anti-bacterial Drugs, 2014); length of hospital stay; and adverse events probably related to the study regimens. Data was extracted preferentially by intention to treat.

Data Analysis and Statistical Methods

Heterogeneity was examined using the χ^2 test ($P \leq 0.1$) and the I^2 test ($I^2 > 50\%$ defining significant inconsistency). Publication bias was assessed using the funnel plot method and Egger's test. Risk ratios (RRs) were calculated for individual trials, with 95% confidence intervals (CIs). Meta-analysis was conducted using the Mantel–Haenszel fixed-effects model. We compared the fixed-effect model to a random-effects model when we observed significant heterogeneity between the trials ($P \leq 0.10$). The results from the fixed-effects model are presented only when there was no significant heterogeneity between trials ($P > 0.1$); otherwise, the results from the random-effects model are presented. Analyses were conducted using Stata 11.0. For studies with multiple treatment groups, we assessed intervention groups for relevance for our review. If more than two groups were relevant, we combined groups to create a single pair-wise comparison.

RESULTS

Study Selection Process

The flow diagram in **Figure 1** shows the detailed screening and selection process applied before including trials in the meta-analysis. We identified a total of 1,749 citations from biomedical databases. After screening all titles and/or abstracts, 67 studies were identified for full text review. Forty-four studies were subsequently excluded for the following reasons: inappropriate comparison arms ($n = 27$); studies on patients in ICU or outpatients ($n = 12$); including HCAP patients ($n = 2$); including children ($n = 1$); same database as studies already included ($n = 1$); conference abstracts ($n = 2$). Twenty-two full-text publications involving 6,235 patients were ultimately identified (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005; Chang et al., 2006; Xu et al., 2006; Zhang et al., 2006; Lin et al., 2007; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Lee et al., 2012; Liu et al., 2012; Postma et al., 2015).

Study Characteristics

The main characteristics of the included trials are shown in **Table 1**. The trials were carried out between 1997 and 2013 in more than 25 countries. With a mean or median age between 47 and 77 years, the patients enrolled were mainly Caucasian and Asian and mostly from European counties, China, and the United States (US). Data on the comparison of respiratory fluoroquinolone monotherapy with β-lactam monotherapy was available in two trials (Leophonte et al., 2004; Postma et al., 2015), β-lactam–macrolide combination therapy in 16 trials (Frank et al., 2002; Zervos et al., 2004; Portier et al., 2005; Xu et al.,

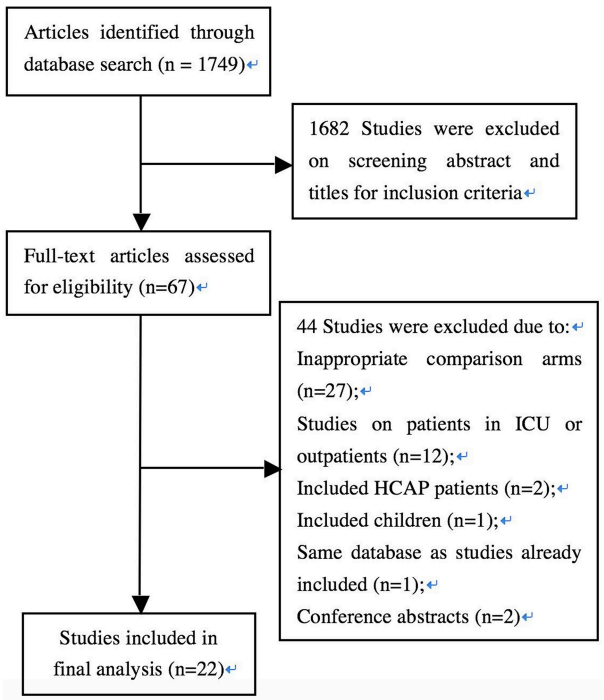

FIGURE 1 | Flow diagram of the selection of studies for inclusion in the meta-analysis.

2006; Zhang et al., 2006; Lin et al., 2007; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Lee et al., 2012; Liu et al., 2012), and β-lactam with or without macrolide (β-lactam ± macrolide) in five trials (Finch et al., 2002; Lode et al., 2002; Erard et al., 2004; Welte et al., 2005; Chang et al., 2006). Patients received sequential intravenous to oral or intravenous antibiotics in 20 trials (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Zervos et al., 2004; Welte et al., 2005; Chang et al., 2006; Xu et al., 2006; Zhang et al., 2006; Lin et al., 2007; Zhao and Chen, 2007; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Lee et al., 2012; Liu et al., 2012; Postma et al., 2015). Treatment was given orally initially in two trials (Leophonte et al., 2004; Portier et al., 2005). We did not find publication bias in the performed analyses.

Sequence generation (specified rule for allocating interventions to participants based on some random process) (Higgins and Altman, 2011a) was adequate in 6 studies (Frank et al., 2002; Welte et al., 2005; Lin et al., 2007; Li et al., 2009; Lee et al., 2012; Postma et al., 2015) and no information was available for other studies. With numbered sachets, only Léophonte's study (Leophonte et al., 2004) reported adequate allocation concealment (steps taken to secure strict implementation of random assignments by preventing foreknowledge of the forthcoming allocations) (Higgins and Altman, 2011a). Insufficient information was available for the other studies. One trial (Leophonte et al., 2004) was double-blinded and the remaining were open label. Details of the incomplete data for each outcome will be discussed in the following sections. We did not find any specific concerns over selective reporting. For other potential source of bias, we found that seven studies (Lode et al., 2002; Erard et al., 2004; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005; Lin et al., 2007; Lee et al., 2012) were sponsored by pharmaceutical companies, which might generate bias in the assessment of outcomes. Besides, one study (Postma et al., 2015) was a cluster-randomized, crossover trial comparing treatment strategies assigned to hospitals in defined study periods as the unit of randomization. Analyses in this study took into account cluster-period effects and center effects.

Mortality

Nine trials provided mortality outcomes (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005; Postma et al., 2015). In total, 114 (5.2%) of the 2,198 patients in the respiratory fluoroquinolone group and 191 (7.2%) of the 2,670 patients in the comparator group died during the course of the studies. A non-significant trend toward an advantage to the respiratory fluoroquinolone group was observed (RR 0.82, 95% CI 0.65–1.02) (**Figure 2**). No heterogeneity was observed ($I^2 = 0\%$).

Data about mortality of patients with β-lactam monotherapy was available for 2 trials (Leophonte et al., 2004; Postma et al., 2015) and no significant difference was found (RR 0.99, 95% CI 0.72–1.35). The non-significant advantage of the respiratory fluoroquinolone group was seen in the patients with β-lactam–macrolide combination therapy from 4 trials (RR 0.81, 95% CI 0.62–1.06) (Frank et al., 2002; Zervos et al., 2004; Portier et al., 2005; Postma et al., 2015). However, mortality rate was significantly lower in the respiratory fluoroquinolone group among patients with β-lactam ± macrolide regimen from 4 trials (RR 0.56, 95% CI 0.33–0.98) (Finch et al., 2002; Lode et al., 2002; Erard et al., 2004; Welte et al., 2005).

The same non-significant advantage of the respiratory fluoroquinolone group was seen when we excluded the cluster-randomized cross-over trial (RR = 0.70, 95% CI 0.46–1.07) (Postma et al., 2015).

Clinical Treatment Success

Data about clinical treatment success in the intention-to-treat population were available for 8 trials (Frank et al., 2002; Lode et al., 2002; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005; Zhang et al., 2006; Lin et al., 2007). Overall, treatment with respiratory fluoroquinolone was successful for 804 (80.9%) of the 994 patients. Treatment with comparator antibiotics was successful for 775 (78.4%) of the 988 patients. Meta-analysis showed that there was no significant difference (RR 1.03, 95% CI 0.99–1.08) (**Figure 3**). No heterogeneity was observed ($I^2 = 0\%$). The same conclusion was drawn from separate analyses of the studies on β-lactam–macrolide combination therapy (RR= 1.05, 95% CI 0.99–1.11) (Frank et al., 2002; Zervos et al., 2004; Portier et al., 2005; Zhang et al., 2006; Lin et al., 2007) and β-lactam ± macrolide regimen (RR 1.01, 95% CI 0.92–1.10) (Lode et al., 2002; Welte et al., 2005). Only one study

TABLE 1 | Study characteristics.

Study	Location	Population	Enrolled patients	Age (years)	Drug tested		Duration (d)	Funding source
			FQ/β±M	FQ/β±M	FQ	β±M		
Chang et al., 2006	China	Asian	41/41	47 (18–70)	Sequential i.v. levofloxacin 400 mg OD followed by p.o. levofloxacin 100 mg t.i.d.	Sequential i.v. cefuroxime 1,500 mg b.i.d. followed by p.o. cefuroxime axetil 500 mg b.i.d. ± p.o. roxithromycin 150 mg b.i.d.	7–10	NS
Erard et al., 2004	Switzerland	Caucasian	79/37	77 (24–92)/77 (26–95)	p.o. levofloxacin 500 mg q12h	Sequential i.v. and p.o. ceftriaxone 2 g OD ± i.v./p.o. clarithromycin 500 mg q12h	7–10	Aventis
Finch et al., 2002	Belgium, France, Germany, Greece, Israel, South Africa, Spain, Switzerland, Russia, UK	Mixed	301/321	55.2 ± 20.6/55.9 ± 19.6	Sequential i.v. and p.o. moxifloxacin 400 mg OD	Sequential i.v. 1.2 g and p.o. 625 mg co-amoxiclav t.i.d. ± i.v./p.o. clarithromycin 500 mg b.i.d.	7–14	NS
Frank et al., 2002	USA	Mixed	115/121	67.8 ± 13.11/67.3 ± 13.17	i.v./p.o. levofloxacin 500 mg OD	i.v. ceftriaxone 1 g OD + i.v. azithromycin 500 mg OD	≥10	NS
Gao et al., 2009	China	Asian	40/38	55.2 ± 12.3/54.3 ± 13.6	Sequential i.v. and p.o. moxifloxacin 400 mg OD	i.v. cefuroxime 2g b.i.d. + p.o. azithromycin 500 mg OD	7–14	NS
Han et al., 2010	China	Asian	40/40	47.95 ± 15.13/47.85 ± 15.85	i.v. moxifloxacin 400 mg OD	i.v. ceftriaxone 3 g OD + i.v. azithromycin 500 mg OD	7–10	NS
Huang et al., 2008	China	Asian	119/65	71.4 ± 5.0/72.7 ± 5.4	i.v. moxifloxacin 400 mg OD or Sequential i.v. and p.o. moxifloxacin 400 mg OD	i.v. cefuroxime 3 g b.i.d. + i.v. azithromycin 500 mg OD	7–10	NS
Lee et al., 2012	Korea	Asian	20/20	54 ± 20/53 ± 16	Sequential i.v. and p.o. levofloxacin 750 mg OD	i.v. ceftriaxone 2 g OD + p.o. azithromycin 500 mg OD, followed by p.o. cefpodoxime 200 mg/D	NS	Daiichi-Sankyo Korea
Leophonte et al., 2004	France, Poland, South Africa	Mixed	167/153	53.3 ± 20.4/55.3 ± 19.8	p.o. gemifloxacin 320 mg OD	p.o. amoxicillin/clavulanate 1 g/125 mg t.i.d.	7–10	NS
Li et al., 2009	China	Asian	40/35	55.1 ± 12.5/54.2 ± 13.1	i.v. levofloxacin 500 mg OD	i.v. cefuroxime 2g b.i.d. + azithromycin 500 mg OD	7–14	NS
Lin et al., 2007	Taiwan	Asian	26/24	65.3 ± 13.2/71.0 ± 11.4	Sequential i.v. and p.o. levofloxacin 500 mg OD	Sequential i.v. 500 mg/100 mg and p.o. 250 mg/125 mg amoxicillin/clavulanate q8h + p.o. azithromycin 500 mg q12h	7–14	Daiichi
Liu et al., 2012	China	Asian	33/33	73 ± 11.48/72 ± 8.78	i.v. moxifloxacin 400 mg OD	i.v. cefoperazone/sulbactam 2.5 g b.i.d. + i.v. azithromycin 0.5 g OD	7–14	NS

(Continued)

TABLE 1 | Continued

Study	Location	Population	Enrolled patients	Age (years)	Drug tested		Duration (d)	Funding source
			FQ/β±M	FQ/β±M	FQ	β±M		
Lode et al., 2002	US, Poland, Canada, Germany, Italy, UK, Australia, Austria, Belgium, Guatemala, Hungary, Lebanon, Philippines, Singapore, Switzerland	Mixed	172/173	59.5 ± 17.7/58.2 ± 18.7	p.o. gemifloxacin 320 mg OD	Sequential i.v. ceftriaxone 2 g OD followed by p.o. cefuroxime 500 mg b.i.d. ± macrolide	7–14	GSK
Portier et al., 2005	France	Caucasian	174/175	59.3 ± 17.9/62.4 ± 18.0	p.o. moxifloxacin 400 mg OD	p.o. amoxicillin-clavulanate 1,000/125 mg t.i.d. + p.o. roxithromycin 150 mg b.i.d.	10	Bayer
Postma et al., 2015	the Netherlands	Caucasian	888/1395	71 ± 14.81/70 ± 14.87	moxifloxacin or levofloxacin	Beta-lactam (amoxicillin, amoxicillin plus clavulanate, or a third-generation cephalosporin) monotherapy and combined with macrolide (azithromycin, erythromycin, or clarithromycin)	NS	NS
Shao et al., 2008	China	Asian	199/199	47.43 ± 18.94/51.50 ± 19.95	Sequential i.v. and p.o. levofloxacin 500 mg OD	Sequential i.v. cefuroxime 1500 mg b.i.d. followed by p.o. cefuroxime axetil 500 mg b.i.d. + p.o. azithromycin 500 mg t.i.d.	10–14	NS
Welte et al., 2005	Germany, France, Greece, Lithuania, and Poland	Caucasian	200/197	NS	Sequential i.v. and p.o. moxifloxacin 400 mg OD	i.v. ceftriaxone 2 g OD ± i.v. erythromycin 1 g q6-8h	7–14	Bayer Vital GmbH
Xu et al., 2006	China	Asian	20/20	NS	i.v. moxifloxacin 400mg OD	i.v. cefoperazone 2 g b.i.d. + i.v. azithromycin 0.5 g OD	7–14	NS
Yang and Zhang, 2009	China	Asian	50/50	72.9/73.3	i.v. moxifloxacin 400 mg OD	i.v. ceftriaxone 2 g OD + i.v. azithromycin 0.5 g OD	7	NS
Zervos et al., 2004	US, Canada, and Europe	Mixed	112/107	72.8 ± 13.6/70.7 ± 13.5	i.v. levofloxacin 500 mg OD	i.v. ceftriaxone 1 g OD + i.v. azithromycin 500 mg OD	7–14	Pfizer and Pliva
Zhang et al., 2006	China	Asian	50/50	58.1 ± 11.7/56.8 ± 12.4	i.v. levofloxacin 300 mg b.i.d.	i.v. ceftriaxone 1 g b.i.d. + p.o. azithromycin 500 mg OD	7–14	NS
Zhao and Chen, 2007	China	Asian	30/25	55.2 ± 12.3/54.3 ± 13.6	i.v. levofloxacin 500 mg OD	i.v. cefuroxime 2.25 g b.i.d. + p.o. azithromycin 500 mg OD	7–14	NS

FQ, fluoroquinolone; β±M, β-lactam with or without macrolide; NS, not specified; i.v., intravenous; p.o., oral; OD, once daily; b.i.d., twice daily; t.i.d., three times daily.

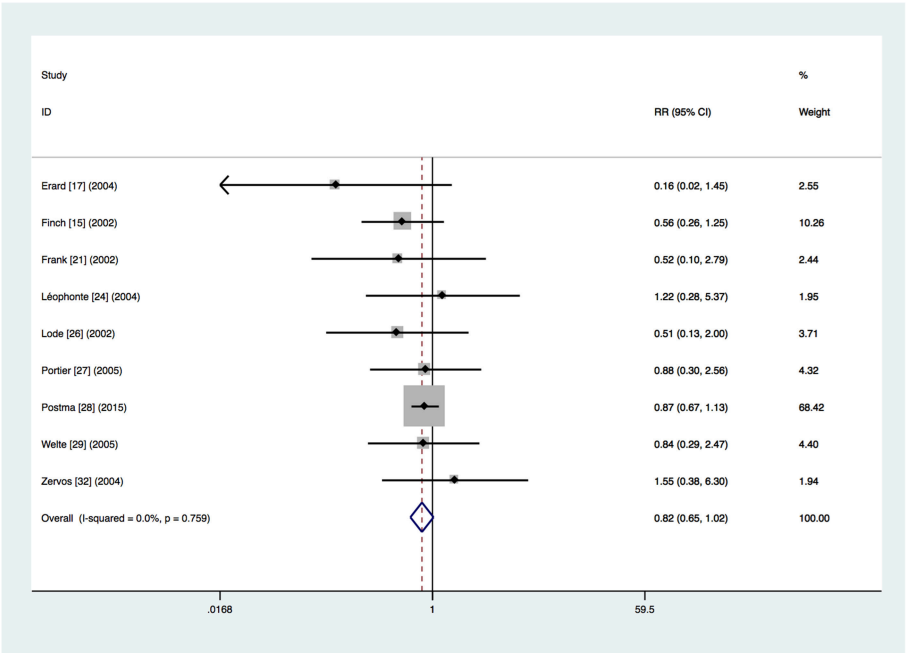

FIGURE 2 | Mortality for respiratory fluoroquinolone monotherapy vs. β-lactam with or without macrolide. A fixed-effect Mantel–Haenszel (M–H) meta-analysis is shown with results presented as risk ratios with 95% confidence intervals (CIs).

(Leophonte et al., 2004) used β-lactam monotherapy and thus a combined analysis could not be performed. No significant difference was found in studies where treatment was given orally (RR 1.05, 95% CI 0.98–1.12) (Leophonte et al., 2004; Portier et al., 2005) or initially intravenously (RR 1.02, 95% CI 0.97–1.08) (Frank et al., 2002; Lode et al., 2002; Zervos et al., 2004; Welte et al., 2005; Zhang et al., 2006; Lin et al., 2007). No significant difference was found in the trials funded by pharmaceutical companies (RR 1.03, 95% CI 0.97–1.09) (Lode et al., 2002; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005; Lin et al., 2007) or not (RR 1.04, 95% CI 0.97–1.11) (Frank et al., 2002; Leophonte et al., 2004; Zhang et al., 2006).

Eleven trials provided data about clinical treatment success in the clinically evaluable population (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005; Zhang et al., 2006; Lin et al., 2007; Lee et al., 2012). The clinical treatment success was 91.3% (1,048 of the 1,148 patients) in the respiratory fluoroquinolone group and 88.9% (984 of the 1,107 patients) in the comparator antibiotics group. Meta-analysis showed that there was no significant difference (RR 1.03, 95% CI 0.999–1.055) (**Figure 4**). No significant heterogeneity was observed ($I^2 = 2.1\%$). The same conclusion was drawn from separate analyses of the studies on β-lactam–macrolide combination therapy (RR 1.00, 95% CI 0.96–1.05) (Frank et al., 2002; Zervos et al., 2004; Portier et al., 2005; Zhang et al., 2006; Lin et al., 2007; Lee et al., 2012) and β-lactam ± macrolide regimen (RR 1.02, 95% CI 0.97–1.08) (Finch et al., 2002; Lode et al., 2002; Erard et al., 2004; Welte et al., 2005). Only one study used β-lactam monotherapy (Leophonte et al., 2004). No significant difference was found in studies where treatment was given orally (RR 1.03, 95% CI 0.97–1.09) (Leophonte et al., 2004; Portier et al., 2005) or initially intravenously (RR 1.03, 95% CI 0.996–1.059) (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Zervos et al., 2004; Welte et al., 2005; Zhang et al., 2006; Lin et al., 2007; Lee et al., 2012). No significant difference was found in the trials funded by pharmaceutical companies (RR 1.00, 95% CI 0.96–1.04) (Lode et al., 2002; Erard et al., 2004; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005; Lin et al., 2007; Lee et al., 2012). However, the advantage of respiratory fluoroquinolone was statistically significant in the studies not funded by pharmaceutical companies (RR 1.06, 95% CI 1.02–1.10) (Finch et al., 2002; Frank et al., 2002; Leophonte et al., 2004; Zhang et al., 2006).

It was unclear whether intention-to-treat or per-protocol analysis was used in ten studies, which did not refer to dropouts or reported the total number of dropouts but did not give the numbers per study arm (Chang et al., 2006; Xu et al., 2006; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Liu et al., 2012). The clinical treatment success was 93.7% (565 of the 603 patients) in the respiratory fluoroquinolone group and 89.5% (479 of the 535 patients) in the comparator antibiotics group. Heterogeneity was detected ($I^2 = 38.7\%$, $P = 0.10$) and meta-analysis done by the random-effects model showed no significant difference (RR 1.04, 95% CI 0.99–1.09) (**Figure 5**). The advantage of respiratory fluoroquinolone was

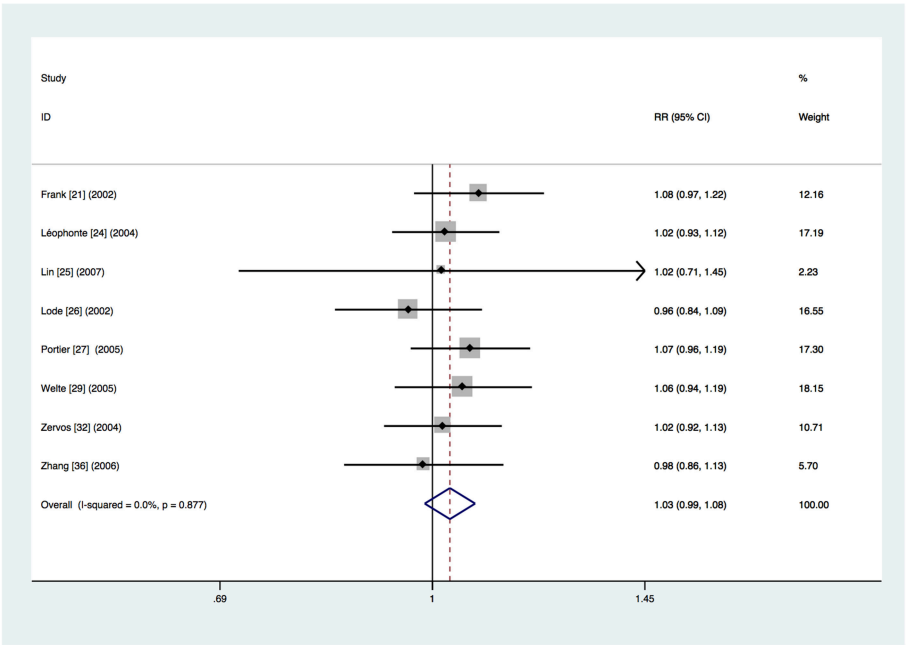

FIGURE 3 | Clinical treatment success analysis based on intention-to-treat population.

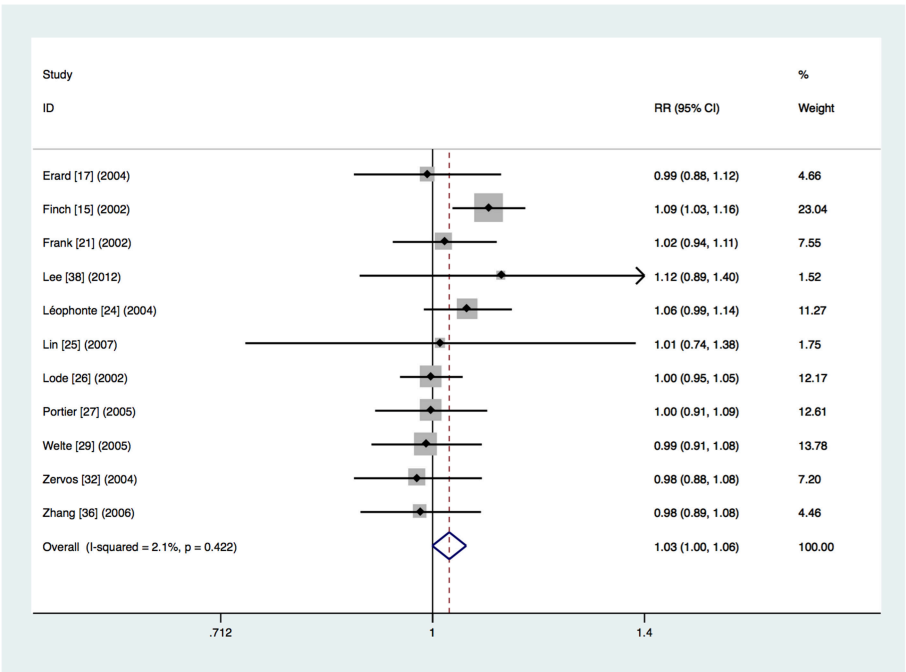

FIGURE 4 | Clinical treatment success analysis based on clinically evaluable population.

statistically significant when compared with β-lactam–macrolide combination therapy (RR 1.05, 95% CI 1.01–1.09) (Xu et al., 2006; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Liu et al., 2012) and the heterogeneity was reduced in this analysis (I^2 = 25.8%, P = 0.21). Only one study used β-lactam ± macrolide regimen (Chang et al., 2006) and no trials used β-lactam monotherapy. Treatment was given

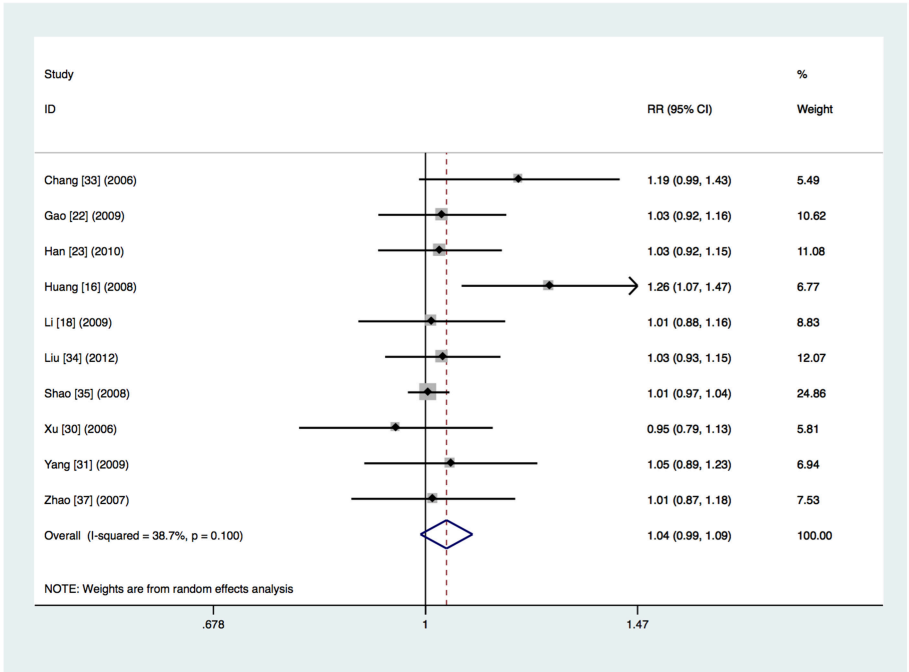

FIGURE 5 | Clinical treatment success analysis for the studies in which it was unclear whether intention-to-treat or per-protocol analysis was used.

initially intravenously in all trials. Not any study was funded by pharmaceutical companies.

Microbiological Treatment Success

Eighteen studies reported microbiological treatment success outcomes (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Chang et al., 2006; Xu et al., 2006; Zhang et al., 2006; Lin et al., 2007; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Lee et al., 2012). In the total microbiologically evaluable population, 513 (88.8%) of the 578 patients/isolates in the respiratory fluoroquinolone group and 462 (85.2%) of the 542 patients/isolates in the comparator group achieved eradication or presumed eradication of the baseline pathogens. The most common pathogens were *S. pneumoniae*, *H. influenza*, and *M. pneumoniae*. Details about drug resistance were reported in 9 trials (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Zhang et al., 2006; Postma et al., 2015). For respiratory fluoroquinolone, only one *S. aureus* isolate resistant to levofloxacin was found. *S. pneumoniae* strains resistant to the comparator antibiotics were more commonly found. Resistance was more prominent among macrolides than among β-lactams.

There was no significant difference in the overall microbiological treatment success rates between the two groups (RR 1.04, 95% CI 0.997–1.092). No significant heterogeneity was observed (I^2 = 10.3%). The same conclusion was drawn from separate analyses of the studies on β-lactam–macrolide combination therapy (RR 1.05, 95% CI 0.98–1.12) (Frank et al., 2002; Zervos et al., 2004; Portier et al., 2005; Xu et al., 2006; Zhang et al., 2006; Lin et al., 2007; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Lee et al., 2012) and β-lactam ± macrolide regimen (RR 1.07, 95% CI 0.99–1.15) (Finch et al., 2002; Lode et al., 2002; Chang et al., 2006). Only one study used β-lactam monotherapy (Leophonte et al., 2004). No significant difference was found in studies where treatment was given orally (RR 0.98, 95% CI 0.88–1.10) (Leophonte et al., 2004; Portier et al., 2005). In studies where treatment was given initially intravenously, the advantage of respiratory fluoroquinolone was statistically significant (RR 1.05, 95% CI 1.003–1.108) (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Zervos et al., 2004; Chang et al., 2006; Xu et al., 2006; Zhang et al., 2006; Lin et al., 2007; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Lee et al., 2012).

In addition, there was no significant difference between the respiratory fluoroquinolone group and the comparator group for the microbiological treatment success rates of *S. pneumoniae* (343 isolates, RR 0.99, 95% CI 0.85–1.17) (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Xu et al., 2006; Zhang et al., 2006; Lin et al., 2007; Han et al., 2010; Lee et al., 2012), *H. influenzae* (113 isolates, RR 1.04, 95% CI 0.87–1.25) (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Leophonte et al., 2004; Zervos et al., 2004; Xu et al., 2006; Zhang et al., 2006; Lin et al., 2007; Han et al., 2010), *M. pneumoniae* (77 isolates, RR 1.08, 95% CI 0.96–1.23) (Finch et al., 2002; Lode et al., 2002; Han et al., 2010; Lee et al., 2012), *C. pneumoniae*

(41 isolates, RR 1.03, 95% CI 0.83–1.27) (Finch et al., 2002; Lode et al., 2002; Han et al., 2010) and *Legionella* species (21 isolates, RR 0.99, 95% CI 0.60–1.63) (Finch et al., 2002; Lode et al., 2002; Leophonte et al., 2004).

Length of Hospital Stay

Data about the length of stay in hospital were available in 9 trials (Finch et al., 2002; Lode et al., 2002; Erard et al., 2004; Zervos et al., 2004; Welte et al., 2005; Lin et al., 2007; Shao et al., 2008; Li et al., 2009; Postma et al., 2015). Four trials provided the median duration of hospital stay and 0–2 days less duration was found in the respiratory fluoroquinolone group (Lode et al., 2002; Erard et al., 2004; Welte et al., 2005; Postma et al., 2015). Six trials provided the mean duration of hospital stay and no significant difference was found (SMD −0.06, 95% CI −0.22 to 0.11) (Finch et al., 2002; Zervos et al., 2004; Welte et al., 2005; Lin et al., 2007; Shao et al., 2008; Li et al., 2009). Among these studies, one trial provided both the median and the mean duration (Welte et al., 2005). Using the statistic methods recommended in the Cochrane Hand-book (Higgins and Altman, 2011b), we calculated the mean duration for all trials and performed an overall meta-analysis. No significant difference was found (SMD −0.06, 95% CI −0.16 to 0.04). Heterogeneity was moderate ($I^2 = 45.6\%$). However, the advantage of respiratory fluoroquinolone was statistically significant when compared with β-lactam ± macrolide regimen (SMD −0.18, 95% CI −0.28 to −0.07) (Finch et al., 2002; Lode et al., 2002; Erard et al., 2004; Welte et al., 2005) and the heterogeneity was reduced in this analysis ($I^2 = 9.7\%$). No significant difference was found when respiratory fluoroquinolone was compared with β-lactam–macrolide combination therapy (SMD 0.03, 95% CI −0.06 to 0.11) (Zervos et al., 2004; Lin et al., 2007; Shao et al., 2008; Li et al., 2009; Postma et al., 2015). Data of patients with β-lactam monotherapy was only available in one trial (Postma et al., 2015).

Adverse Events

All but two trials reported on drug-related adverse outcomes. One trial did not refer to adverse events (Lin et al., 2007). One trial reported on complications while data on drug-related adverse outcomes was unavailable (Postma et al., 2015). The majority of the adverse events were mild to moderate. The most commonly studied adverse effects were gastrointestinal events (including nausea, diarrhea and vomiting) and liver function abnormalities. However, the definition of gastrointestinal events differed, some including all the three symptoms (nausea, diarrhea and vomiting) and some nausea alone, thereby excluding an accurate comparison for each symptom alone. QTc prolongation was reported in one trial with one patient in the co-amoxiclav ± clarithromycin group.

The advantage of respiratory fluoroquinolone was statistically significant on the adverse events (RR 0.87, 95% CI 0.77–0.97). No significant heterogeneity was observed ($I^2 = 25.9\%$). The same conclusion was drawn from analysis of the studies on serious adverse events (RR 0.67, 95% CI 0.51–0.88) (Finch et al., 2002; Frank et al., 2002; Leophonte et al., 2004; Zervos et al., 2004; Portier et al., 2005; Welte et al., 2005). The percentage of patients who were withdrawn from the trials because of adverse events was not significantly different between the two groups (RR 0.87, 95% CI 0.59–1.30) (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Zervos et al., 2004; Welte et al., 2005; Chang et al., 2006; Liu et al., 2012). Respiratory fluoroquinolone was associated with significantly fewer adverse events compared with β-lactam–macrolide combination therapy (RR 0.74, 95% CI 0.61–0.90) (Frank et al., 2002; Zervos et al., 2004; Portier et al., 2005; Xu et al., 2006; Zhang et al., 2006; Zhao and Chen, 2007; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Han et al., 2010; Lee et al., 2012; Liu et al., 2012). No significant difference was found when respiratory fluoroquinolone was compared with β-lactam ± macrolide regimen (RR 0.99, 95% CI 0.74–1.34) (Finch et al., 2002; Lode et al., 2002; Erard et al., 2004; Welte et al., 2005; Chang et al., 2006). Only one study used β-lactam monotherapy (Leophonte et al., 2004).

Gastrointestinal events were reported in 16 studies and were significantly less common in the respiratory fluoroquinolone group (RR 0.63, 95% CI 0.43 to 0.94) (Finch et al., 2002; Frank et al., 2002; Lode et al., 2002; Erard et al., 2004; Leophonte et al., 2004; Portier et al., 2005; Welte et al., 2005; Chang et al., 2006; Xu et al., 2006; Zhang et al., 2006; Huang et al., 2008; Shao et al., 2008; Gao et al., 2009; Han et al., 2010; Lee et al., 2012; Liu et al., 2012). Non-significant advantage of respiratory fluoroquinolone was found with regard to liver function abnormalities (RR 0.73, 95% CI 0.52 to 1.03) (Finch et al., 2002; Lode et al., 2002; Leophonte et al., 2004; Welte et al., 2005; Zhang et al., 2006; Zhao and Chen, 2007; Shao et al., 2008; Gao et al., 2009; Li et al., 2009; Yang and Zhang, 2009; Lee et al., 2012).

DISCUSSION

This systematic review with meta-analysis compared the efficacy and safety of respiratory fluoroquinolone monotherapy and β-lactam with or without macrolide for non-ICU hospitalized CAP patients. A non-significant trend toward an advantage to respiratory fluoroquinolone was observed on overall mortality. No significant difference was found between the two strategies in clinical success, microbiological treatment success, and length of stay. The advantage of respiratory fluoroquinolone was statistically significant in the drug-related adverse events. The advantage of respiratory fluoroquinolone in clinical treatment success was statistically significant in the studies not funded by pharmaceutical companies based on the clinically evaluable population (RR 1.06, 95% CI 1.02–1.10) and the advantage in microbiological treatment success was statistically significant in the studies where treatment was given initially intravenously (RR 1.05, 95% CI 1.003–1.108). The results were consistent with those of the primary analysis for the subgroup of β-lactam–macrolide combination therapy except for the clinical success based on the data that it was unclear whether intention-to-treat or per-protocol analysis was used (RR 1.05, 95% CI 1.01–1.09). Analysis was available only in mortality for the subgroup of β-lactam monotherapy and no significant difference was found. For the subgroup of β-lactam ± macrolide regimen, respiratory fluoroquinolone was associated with significantly lower mortality and less length of stay, while no significant difference was found in clinical treatment success, microbiological treatment success and adverse events.

An earlier meta-analysis performed by Vardakas et al. (2008) investigated whether respiratory quinolone monotherapy was superior to other recommended antimicrobial regimens, including combination therapy consisting of a macrolide and β-lactam as well as monotherapy (macrolide, ketolide, or β-lactam alone), for the treatment of adults with CAP. While no significant difference was found in mortality, clinical success rates were significantly higher and adverse events were significantly fewer with fluoroquinolone monotherapy. However, we found no significant difference in the overall clinical treatment success. In our meta-analysis, we focused on direct comparison of respiratory fluoroquinolone monotherapy and β-lactam with or without macrolide for non-ICU hospitalized CAP patients, precluding the interference from outpatients or patients in ICU and the interference from other drugs. Furthermore, we included new trials performed in recent years, providing greater statistical confidence for our meta-analysis.

The moderate total mortality rates in the two groups of our meta-analysis (5.2% and 7.2%) supports the opinion that the patients admitted to non-ICU hospital wards are associate with moderate risk of death (Mandell et al., 2007; Lim et al., 2009). A non-significant trend toward an advantage to the respiratory fluoroquinolone group was observed and more RCTs are needed to further verify the result.

Overall, no significant difference was found in clinical treatment success. The advantage of respiratory fluoroquinolone was statistically significant in some subgroup analyses. However, we noticed that the advantage was not obvious (RR = 1.06 and RR = 1.05). Therefore, we considered that the advantages of respiratory fluoroquinolone in these subgroup analyses were limited in clinical significance.

Drug resistance was found more prominent in the comparator antibiotics and most commonly among macrolides, which was in correspondence with previous surveillance (Mandell et al., 2007; Ho et al., 2009; Lim et al., 2009). There was no significant difference in the microbiological treatment success. However, the amount of patients enrolled in the analysis was limited (578/542 patients/isolates) and the patients included in the analysis for atypical pathogens were mainly from European countries. Previous surveillance results showed that the resistance of *M. pneumoniae* to macrolides in Asian countries was significantly higher than in the European or North American countries (Mandell et al., 2007; Lim et al., 2009; Mikasa et al., 2016; Cao et al., 2018). Since the drug resistance pattern differs greatly in different areas and countries, more RCTs with large scale in different areas are needed to verify the above conclusion.

When respiratory fluoroquinolone was compared with β-lactam–macrolide combination therapy, no significant difference was found in mortality, clinical treatment success, microbiological treatment success and length of stay. Respiratory fluoroquinolone was associated with fewer adverse events. When respiratory fluoroquinolone was compared with β-lactam monotherapy, no significant difference was found in mortality. Because of the lack of studies in this subgroup, analyses for other outcomes were not available. This may be because researchers used β-lactam monotherapy mainly in outpatients with low severity and usually added macrolides for hospitalized patients with moderate to severe pneumonia. More studies or detailed data comparing respiratory fluoroquinolone with β-lactam monotherapy in hospitalized CAP patients under supervision are needed. In the studies with β-lactam ± macrolide regimen as control group, respiratory fluoroquinolone was associated with significantly lower mortality rate and less length of stay. No significant difference was found in clinical treatment success, microbiological treatment success and adverse events. As the comparator regimens in these studies were not exactly the same, the results of this subgroup analysis might introduce more bias and thus provided relatively less statistical confidence.

There were several limitations in our meta-analysis. First, our findings may be affected by the quality of trials included in the analysis. Sequence generation was adequate in 6 studies. Only one trial was double-blinded, and one trial reported adequate allocation concealment. A sensitivity analysis was performed including only trials that reported adequate sequence generation. The results were consistent with those of the primary analysis except for overall adverse events rate, which indicated non-significant advantage of the respiratory fluoroquinolone group. Second, the quantity of studies included in some subgroup analyses was small, resulting in limited statistical confidence. Third, we failed to perform a comprehensive analysis for β-lactam monotherapy because of the lack of studies comparing respiratory fluoroquinolone with it. Finally, seven studies were sponsored by pharmaceutical companies, which might generate bias in the assessment of outcomes. Sensitivity analyses limited to industry-funded and not industry-funded studies were performed. The results showed that for the clinical treatment success in the clinically evaluable population, the advantage of respiratory fluoroquinolone was statistically significant in the studies not funded by pharmaceutical companies but limited in clinical significance. For the overall adverse events, no significant difference was found in the studies not funded by pharmaceutical companies. Other analyses indicated similar findings with the primary analyses.

In conclusion, despite the limitations of our meta-analysis, we conclude that respiratory fluoroquinolone monotherapy has similar efficacy and favorable safety compared with β-lactam with or without macrolide for non-ICU hospitalized CAP patients. Since the limitation of region, quantity and quality of included studies, more RCTs with large scale and high quality are needed to verify the above conclusion.

AUTHOR CONTRIBUTIONS

HF, SL, and XT conceived and designed the studies. SL and XT carried out the literature search, extracted data, assess the risk of bias of the RCTs. YM, DW, JH, LZ, MW, LW, and TL helped conduct the analyses. SL wrote the manuscript. All authors reviewed the manuscript.

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REFERENCES

Aldred, K. J., Kerns, R. J., and Osheroff, N. (2014). Mechanism of quinolone action and resistance. *Biochemistry* 18, 1565–1574. doi: 10.1021/bi5000564

Cao, B., Huang, Y., She, D. Y., Cheng, Q. J., Fan, H., Tian, X. L., et al. (2018). Diagnosis and treatment of community-acquired pneumonia in adults: 2016 clinical practice guidelines. *Clin. Respir. J.* 12, 1320–1360. doi: 10.1111/crj.12674

Chang, H. Q., Wang, J. X., and Xu, S. P. (2006). Observation of curative effect of levofloxacin on community-acquired pneumonia (Chinese). *Beijing Med. J.* 28:753. doi: 10.3969/j.issn.0253-9713.2006.12.021

Erard, V., Lamy, O., Bochud, P.-Y., Bille, J., Cometta, A., and Calandra, T. (2004). Full-course oral levofloxacin for treatment of hospitalized patients with community-acquired pneumonia. *Eur. J. Clin. Microbiol. Infect. Dis.* 23, 82–88. doi: 10.1007/s10096-003-1060-x

Finch, R., Schürmann, D., Collins, O., Kubin, R., McGivern, J., Bobbaers, H., et al. (2002). Randomized controlled trial of sequential intravenous (iv) and oral moxifloxacin compared with sequential iv and oral co-amoxiclav with or without clarithromycin in patients with community-acquired pneumonia requiring initial parenteral treatment. *Antimicrob. Agents Chemother.* 46, 1746–1754. doi: 10.1128/AAC.46.6.1746-1754.2002

Fisher, J. F., Meroueh, S. O., and Mobashery, S. (2005). Bacterial resistance to beta-lactam antibiotics: compelling opportunism, compelling opportunity. *Chem. Rev.* 105, 395–424. doi: 10.1021/cr030102i

Frank, E., Liu, J., Kinasewitz, G., Moran, G. J., Oross, M. P., Olson, W. H., et al. (2002). A multicenter, open-label, randomized comparison of levofloxacin and azithromycin plus ceftriaxone in hospitalized adults with moderate to severe community-acquired pneumonia. *Clin. Ther.* 24, 1292–1308. doi: 10.1016/S0149-2918(02)80034-0

Gao, W. H., Wu, K. S., Lin, Y. H., and Chen, Z. S. (2009). Comparison of moxifloxacin and cefuroxime combined with azithromycin for patients with community-acquired pneumonia (Chinese). *Chin. J. Nosocomiol.* 19, 3105–3107. doi: 10.3321/j.issn:1005-4529.2009.22.041

Han, B., Ning, X. C., and Chai, W. S. (2010). Clinical observation of moxifloxacin versus ceftriaxone combined with azithromycin in the treatment (Chinese). *Chin. Pharm.* 21, 3400–3401. Available online at: http://www.cnki.com.cn/Article/CJFDTotal-ZGYA201036025.htm

Higgins, J., and Altman, D. (2011a). *Chapter 8:Assessing risk of bias in included studies. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011)*. The Cochrane Collaboration. Available online at: www.cochrane-handbook.org (accessed November 25, 2018).

Higgins, J., and Altman, D. E. (2011b). *Chapter 7:Selecting studies and collecting data. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011)*. The Cochrane Collaboration. Available online at: www.cochrane-handbook.org

Ho, P. L., Cheng, V. C., and Chu, C. M. (2009). Antibiotic resistance in community-acquired pneumonia caused by *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus*, and *Acinetobacter baumannii*. *Chest* 136, 1119–1127. doi: 10.1378/chest.09-0285

Hooper, D. C. (2001). Emerging mechanisms of fluoroquinolone resistance. *Emerg. Infect. Dis.* 7, 337–341. doi: 10.3201/eid0702.010239

Huang, G., Jiang, Y. Y., Zhou, Y. J., Ling, X. J., Qiu, T. F., Zhou, Y., et al. (2008). Sequential moxifloxacin therapy in the treatment of elderly patients with community-acquired pnuemonia: clinical evaluation (Chinese). *J. Nanjing Med. Nat. Sci.* 28, 1457–1460. Available online at: http://jnmu.njmu.edu.cn/zr/ch/reader/view_abstract.aspx?file_no=aumn081123&flag=1

Lee, J., Kim, S., Kim, J., Ryu, Y., and Chang, J. (2012). High-dose levofloxacin in community-acquired pneumonia. *Clin. Drug Invest.* 32, 569–576. doi: 10.1007/BF03261911

Leophonte, P., File, T., and Feldman, C. (2004). Gemifloxacin once daily for 7 days compared to amoxicillin/clavulanic acid thrice daily for 10 days for the treatment of community-acquired pneumonia of suspected pneumococcal origin. *Respir. Med.* 98, 708–720. doi: 10.1016/j.rmed.2004.04.007

Li, B. H., Xue, J. M., and Song, J. M. (2009). A comparison between levofloxacin and cefuroxime with azithromycin in community-acquired pneumonia (Chinese). *Chin. Gen. Pract.* 12, 1532–1534. doi: 10.3969/j.issn.1007-9572.2009.16.027

Lim, W. S., Baudouin, S. V., George, R. C., Hill, A. T., Jamieson, C., Le Jeune, I., et al. (2009). BTS guidelines for the management of community acquired pneumonia in adults: update 2009. *Thorax.* 64 (Suppl. 3), iii1–55. doi: 10.1136/thx.2009.121434

Lin, T., Lin, S., Chen, H., Wang, C., Wang, Y., Chang, M., et al. (2007). An open-label, randomized comparison of levofloxacin and amoxicillin/clavulanate plus clarithromycin for the treatment of hospitalized patients with community-acquired pneumonia. *Chang. Gung Med. J.* 30:321.

Liu, L. Y., Xiao, Y. H., Yan, P. K., Xu, J., and Guan, B. E. (2012). Efficacy of moxifloxacin vs. azithromycin plus cefoperazone/sulbactam for community acquired pneumonia (Chinese). *Eval. Anal. Drug Use Hosp. China*. 12, 543-545. Available online at: http://yypf.cbpt.cnki.net/WKD/WebPublication/paperDigest.aspx?paperID=18960cbc-afea-4ddd-b1c6-3e6848ee544b#

Lode, H., File, T. M., Mandell, L., Ball, P., Pypstra, R., Thomas, M., et al. (2002). Oral gemifloxacin versus sequential therapy with intravenous ceftriaxone/oral cefuroxime with or without a macrolide in the treatment of patients hospitalized with community-acquired pneumonia: a randomized, open-label, multicenter study of clinical efficacy and tolerability. *Clin. Ther.* 24, 1915–1936. doi: 10.1016/S0149-2918(02)80088-1

Mandell, L. A., Wunderink, R. G., Anzueto, A., Bartlett, J. G., Campbell, G. D., Dean, N. C., et al. (2007). Infectious diseases society of america/american thoracic society consensus guidelines on the management of community-acquired pneumonia in adults. *Clin. Infect. Dis.* 44 (Suppl. 2), S27–72. doi: 10.1086/511159

Mikasa, K., Aoki, N., Aoki, Y., Abe, S., Iwata, S., Ouchi, K., et al. (2016). JAID/JSC guidelines for the treatment of respiratory infectious diseases: the japanese association for infectious Diseases/Japanese society of chemotherapy - The JAID/JSC guide to clinical management of infectious Disease/Guideline-preparing committee respiratory infectious disease WG. *J. Infect. Chemother.* 22(7 Suppl):S1–S65. doi: 10.1016/j.jiac.2015.12.019

Musher, D. M., and Thorner, A. R. (2014). Community-acquired pneumonia. *N. Engl. J. Med.* 371, 1619–1628. doi: 10.1056/NEJMra1312885

Portier, H., Brambilla, C., Garre, M., Paganin, F., Poubeau, P., and Zuck, P. (2005). Moxifloxacin monotherapy compared to amoxicillin-clavulanate plus roxithromycin for nonsevere community-acquired pneumonia in adults with risk factors. *Eur. J. Clin. Microbiol. Infect. Dis.* 24, 367–376. doi: 10.1007/s10096-005-1347-1

Postma, D. F., van Werkhoven, C. H., van Elden, L. J., Thijsen, S. F., Hoepelman, A. I., Kluytmans, J. A., et al. (2015). Antibiotic treatment strategies for community-acquired pneumonia in adults. *N. Engl. J. Med.* 372, 1312–1323. doi: 10.1056/NEJMoa1406330

Raja, A., Lebbos, J., and Kirkpatrick, P. (2004). Telithromycin. *Nat Rev Drug Discov.* 3(9):733-734. doi: 10.1038/nrd1502

Shao, C. Z., He, L. X., Wang, G. F., Zhou, X., Shen, C., Li, H. P., et al. (2008). A randomized controlled multicentre clinical trial of levofloxacin sequential therapy compared with combination therapy with cefuroxime and azithromycin in patients with community-acquired pneumonia (Chinese). *Chin. J. Infect. Chemother.* 8, 102–106. doi: 10.3321/j.issn:1009-7708.2008.02.005

Suda, K. J., Hicks, L. A., Roberts, R. M., Hunkler, R. J., Matusiak, L. M., and Schumock, G. T. (2018). Antibiotic expenditures by medication, class, and healthcare setting in the united states, 2010-2015. *Clin Infect Dis.* 6, 185–190. doi: 10.1093/cid/cix773

Tenson, T., Lovmar, M., and Ehrenberg, M. (2003). The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *J. Mol. Biol.* 25, 1005–1014. doi: 10.1016/S0022-2836(03)00662-4

Thomas, C. P., Ryan, M., Chapman, J. D., Stason, W. B., Tompkins, C. P., Suaya, J. A., et al. (2012). Incidence and cost of pneumonia in medicare beneficiaries. *Chest.* 142, 973–981. doi: 10.1378/chest.11-1160

Vardakas, K. Z., Siempos, I. I., Grammatikos, A., Athanassa, Z., Korbila, I. P., and Falagas, M. E. (2008). Respiratory fluoroquinolones for the treatment of community-acquired pneumonia: a meta-analysis of randomized controlled trials. *CMAJ* 179, 1269–1277. doi: 10.1503/cmaj.080358

Walsh, C. (2003). Where will new antibiotics come from? *Nat. Rev. Microbiol.* 1, 65-70. doi: 10.1038/nrmicro727

Welte, T., Petermann, W., Schürmann, D., Bauer, T. T., and Reimnitz, P. (2005). Treatment with sequential intravenous or oral moxifloxacin was associated

with faster clinical improvement than was standard therapy for hospitalized patients with community-acquired pneumonia who received initial parenteral therapy. *Clin. Infect. Dis.* 41, 1697–1705. doi: 10.1086/498149

Welte, T., Torres, A., and Nathwani, D. (2012). Clinical and economic burden of community-acquired pneumonia among adults in Europe. *Thorax*. 67, 71–79. doi: 10.1136/thx.2009.129502

Woodhead, M., Blasi, F., Ewig, S., Garau, J., Huchon, G., Ieven, M., et al. (2011). Guidelines for the management of adult lower respiratory tract infections–full version. *Clin. Microbiol. Infect*. 17 (Suppl. 6), E1–E59. doi: 10.1111/j.1469-0691.2011.03672.x

Writing Group of Guidance for Clinical Trials of Anti-bacterial Drugs. (2014). Guidance for clinical trials of anti- bacterial drugs (Chinese). *Chin. J. Clin. Pharmacol*. 30, 844–856. doi: 10.13699/j.cnki.1001-6821.2014.09.030

Xu, S., Xiong, S., Xu, Y., Liu, J., Liu, H., Zhao, J., et al. (2006). Efficacy and safety of intravenous moxifloxacin versus cefoperazone with azithromycin in the treatment of community acquired pneumonia. *J. Huazhong Univ. Sci. Technol. Med. Sci*. 26, 421–424. doi: 10.1007/s11596-006-0411-0

Yang, S. G., and Zhang, S. H. (2009). Efficacy and cost-effectiveness analysis of moxifloxacin hydrochloride and combination of ceftriaxone sodium with azithromycin in the treatment of moderate to severe community acquired pneumonia in elderly patients (Chinese). *Chin. J. New. Drugs* 28, 962–964. Available online at: http://www.wanfangdata.com.cn/details/detail.do?_type=perio&id=zgxyzz200910028

Zervos, M., Mandell, L. A., Vrooman, P. S., Andrews, C. P., McIvor, A., Abdulla, R. H., et al. (2004). Comparative efficacies and tolerabilities of intravenous azithromycin plus ceftriaxone and intravenous levofloxacin with step-down oral therapy for hospitalized patients with moderate to severe community-acquired pneumonia. *Treat. Respir. Med*. 3, 329–336. doi: 10.2165/00151829-200403050-00006

Zhang, J. Y., Yin, C., Dong, B. R., Liu, Y. J., Chen, B., and Yang, R. X. (2006). Multicentre clinical study on levofloxacin intravenous injection for treatment of community-acquired pneumonia (Chinese). *Int. J. Respir*. 26, 245–247. doi: 10.3760/cma.j.issn.1673-436X.2006.04.002

Zhao, Z. M., and Chen, B. Y. (2007). Comparison of levofloxacin alone and cefuroxime combined with azithromycin for patients with community-acquired pneumonia (Chinese). *Chin. J. Nosocomiol*. 17, 594–596. doi: 10.3321/j.issn:1005-4529.2007.05.045

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Clinical Trial Registration and Reporting: Drug Therapy and Prevention of Cardiac-Related Infections

*Lin-Lu Ma[1,2], Yang Qiu[3], Mei-Na Song[4], Yun Chen[1,2], Jian-Xin Qu[1,2], Bing-Hui Li[5], Ming-Juan Zhao[5,6] and Xin-Can Liu[1,2]**

[1] Department of Cardiology, First Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, China, [2] Center for Evidence-Based and Translational Medicine, Henan University of Traditional Chinese Medicine, Zhengzhou, China, [3] Cardiovascular Department, Kaifeng Central Hospital, Kaifeng, China, [4] Department of Nursing, Beijing Tsinghua Changgung Hospital, Beijing, China, [5] Center for Evidence-Based Medicine, Institute of Evidence-Based Medicine and Knowledge Translation, Henan University, Kaifeng, China, [6] Department of Cardiology, The First Affiliated Hospital of Henan University, Kaifeng, China

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Yonggang Zhang,
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UCB Pharma (Belgium), Belgium
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University of Pennsylvania, United States

***Correspondence:**
Xin-Can Liu
liuxincan1965@126.com

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Objective: Clinical trials are the source of evidence. ClinicalTrials.gov is valuable for analyzing current conditions. Until now, the state of drug interventions for heart infections is unknown. The purpose of this study was to comprehensively assess the characteristics of trials on cardiac-related infections and the status of drug interventions.

Methods: The website ClinicalTrials.gov was used to obtain all registered clinical trials on drug interventions for cardiac-related infections as of February 16, 2019. All registration studies were collected, regardless of their recruitment status, research results, and research type. Registration information, results, and weblink-publications of those trials were analyzed.

Results: A total of 45 eligible trials were evaluated and 86.7% of them began from or after 2008 while 91.1% of them adopted interventional study design. Of all trials, 35.6% were completed and 15.6% terminated. Besides, 62.2% of interventional clinical trials recruited more than 100 subjects. Meanwhile, 86.7% of the eligible trials included adult subjects only. Of intervention trials, 65.8% were in the third or fourth phase; 78.1% adopted randomized parallel assignment, containing two groups; 53.6% were masking, and 61.0% described treatment. Moreover, 41.5% of the trials were conducted in North America while 29.3% in Europe. Sponsors for 40.0% of the studies were from the industry. Furthermore, 48.9% of the trials mentioned information on monitoring committees, 24.4% have been published online, and 13.3% have uploaded their results. Drugs for treatments mainly contained antibiotics, among which glycopeptides, β-lactams, and lipopeptides were the most commonly studied ones in experimental group, with the former ones more common. Additionally, 16.2% of the trials evaluated new antimicrobials.

Conclusions: Most clinical trials on cardiac-related infections registered at ClinicalTrials.gov were interventional randomized controlled trials (RCTs) for treatment. Most drugs

focused in trials were old antibiotics, and few trials reported valid results. It is necessary to strengthen supervision over improvements in results, and to combine antibacterial activity with drug delivery regimens to achieve optimal clinical outcomes.

Keywords: cardiac-related infections, Clinicaltrials.gov, clinical trials, trial registration, antibiotics

INTRODUCTION

Infectious diseases are caused by microorganisms (bacteria, viruses, fungi, parasites, etc.) releasing toxins or invading body tissues due to patients' poor constitution and insufficient resistance to pathogens (Friedrich, 2019). These diseases gravely threaten human health, and appropriate drug treatment represents an important management strategy (Zumla et al., 2016). With increasing pressure of anti-infective drug selection, the spread of bacterial resistance and the slowdown of developing new drugs, many previously treatable infectious diseases have now become "incurable" (Hughes, 2014). The problem of bacterial resistance is becoming a serious threat to global public health. An estimated 162,000 people die of multidrug-resistant infections in the United States each year (Burnham et al., 2019). The diagnosis and treatment of infectious diseases are constantly facing new challenges. What are the current status and challenges in the prevention and treatment of common infectious heart diseases and surgical infections in cardiac disease field?

Cardiac-related infections include cardiac infectious diseases, cardiac device infections [permanent pacemakers (PPMS) and implantable cardioverter defibrillators (ICD)], and heart surgery-related infections (Fong, 2009). Many studies have shown microbial infections exhibit many pathogenic behaviors in cardiac-related infections, especially in bacterial and viral infections, which can directly lead to infective endocarditis (IE), myocarditis, pericarditis, cardiac device (permanent pacemakers, and implantable cardioverter defibrillators) implantation infections, and cardio-surgery infections (Fowler et al., 2006; Bennett-Guerrero et al., 2010; Mentzelopoulos et al., 2013; Mayosi et al., 2014; Morillo et al., 2015; Iversen et al., 2019). Many experts have devoted to developing relevant diagnosis and treatment principles and plans for antibiotic prevention (Thornhill et al., 2018). Guidelines released by the European Heart Association and the American Heart Association recommend patients with infective endocarditis on the left side of the heart to accept intravenous antibiotic therapy for 6 weeks (Baddour et al., 2015; Habib et al., 2015). Intravenous therapy during long-term hospitalization may increase the risk of complications, while shorter hospitalization is associated with better outcomes (Boucher, 2019). Besides, the incidence of right-side IE is increasing due to repaired congenital heart disease, the applying of injectable drugs, as well as the implantation of more cardiac devices including cardiac pacemakers, implantable cardioverters, and resynchronization devices (Chirouze et al., 2015). Therefore, given changes in pathogen spectrum and threats from antibiotic resistance, exploring better clinical diagnosis and treatment strategies remains necessary (Nadji et al., 2005).

Clinical trials can provide valid evidence for the safety and efficacy of prevention, diagnosis, and treatment strategies (Califf et al., 2012). ClinicalTrials.gov is a clinical trial database jointly run by the National Library of Medicine (NLM) and the US Food and Drug Administration (FDA) under the National Institutes of Health (NIH). In European Union and the United States, registering all interventional clinical trials is mandatory (Zarin et al., 2011). The International Committee of Medical Journal Editors (ICMJE) announced a policy in which the registration of clinical trials is stipulated to be a precondition for publication (De Angelis et al., 2005). ClinicalTrials.gov is the largest clinical trial registry, with high weekly growth rates for new entries, detailed information on past and present clinical trials, and high transparency and accessibility. Therefore, it could offer even more trials-obtained details than those reported in final peer-reviewed publications (Cihoric et al., 2017).

In recent years, with changes in pathogen spectrum and growing threats from antibiotic resistance, rational use of drugs faces challenges. Therefore, we limited our current analysis to clinical trials accessible at ClinicalTrials.gov to assess the characteristics of cardiac-related infection trials and the status of drug interventions.

METHODS

Accessible records of all clinical trials registered at ClinicalTrials.gov were downloaded, using its advanced search function to search for the terms "cardiac disease, infection," "endocarditis," "pericarditis," "myocarditis," "coronary artery, infection," "aortitis," and "rheumatic heart disease" respectively for "condition or disease" on February 16, 2019. All types of studies were incorporated, including interventional (clinical trials), observational, and expanded studies. Trials of both open (not yet being recruited, recruited) and closed (enrolled through invitation; active, not recruited; suspended; terminated; completed; withdrawn; unknown status) statuses were considered for inclusion. No restrictions were imposed on study results or their enrolled patients' age. All diseases interested in study must be exactly caused by pathogenic microorganism. All included clinical trials must have definitive records on identified anti-infective drugs.

All the following information was extracted from each study: tracking information: actual start date of the study; descriptive information: study type, study phase, study design: interventional study (allocation, intervention model, masking, and primary purpose) and observational study (model, time perspective), number of arms, trial medications, study result, and online linked publications; recruitment information: recruitment status, actual enrollment, estimated completion date of the study, sex/gender,

ages, and location; administrative information: National Clinical Trial (NCT) number, data on monitoring committee (DMC), primary study sponsor, collaborators, and funder type.

All trials were then further subdivided according to classification entry. Descriptive statistics were used to describe qualitative results. Percentage frequency distributions were adopted for categorical data.

RESULTS

As of February 16, 2019, 297, 86, 21, 46, 42, 6, and 31 registered trials were identified on clinicaltrials.gov, using the terms "cardiac disease, infection," "endocarditis," "pericarditis," "myocarditis," "coronary artery, infection," "aortitis," and "rheumatic heart disease," respectively. We excluded duplicated studies and those using non-anti-infective drugs during initial review. In addition, we confirmed that each of the included studies focused diseases directly caused by the infection of pathogenic microorganisms during manual review process. After excluding 484 trials, 45 trials were eventually included (23 focusing on infective endocarditis, three on Chagas heart disease, two on coronary infection, one on parvovirus-mediated cardiomyopathy, one on tuberculous pericarditis, one on children with rheumatic heart disease, 1 on post-resuscitation infection after cardiac arrest and 13 on heart-related device/surgical infections, see **Supplementary Table S1**).

GENERAL CHARACTERISTICS OF THE INCLUDED CLINICAL TRIALS

The enrolled trials were registered between 1999 and 2019, and most (86.7%) of them began between 2008 and 2019. Study duration was within 36 months in more than half of the trials (62.2%), between 36 and 72 months in 26.7% of the trials, and more than 72 months in 11.1% of the trials. Of the eligible trials, 41 (91.1%) were intervention trials and the other four (8.9%) were observational trials. Completed status was dominant in the included trials (n = 16, 35.6%), followed by recruiting status (n = 11, 24.5%). Seven trials (15.6%) were terminated (three lacking funds; two lacking statistical power; one due to business reasons; one due to expired commitment) and one was withdrawn (unable to recruit patients within specified time period. No patients had been enrolled in the study). Most trials actually enrolled a large number of participants; specifically, 62.2% recruited 100 or more participants, 20.0% more than 1,000 participants, and one even recruited 4,000 participants. The included trials were mainly focused on adult patients, that is, 39 (86.7%) only included adult patients, three included individuals less than or equal to 18 years old, and two included subjects younger than 18 years old (**Table 1**).

TABLE 1 | General characteristics of the included trials.

	Number	Percent
Study start date		
Prior to 2008	6	13.3
2008–2010	11	24.5
2011–2013	6	13.3
2014–2016	10	22.2
2017–2019	12	26.7
Length of study time		
0 < L ≤ 36m*	28	62.2
36m < L ≤ 72m	12	26.7
L > 72m	5	11.1
Study type		
Interventional	41	91.1
Observational	4	8.9
Recruitment status		
Not yet recruiting	3	6.7
Recruiting	11	24.5
Enrolling by invitation	1	2.2
Active, not recruiting	3	6.7
Terminated	7	15.6
Completed	16	35.6
Withdrawn	1	2.2
Unknown	3	6.7
Actual enrollment		
<100	17	37.8
100–1,000	17	37.8
1,000–2,000	7	15.6
≥2,000	2	4.4
NP	2	4.4
Ages		
<18 years	2	4.4
Up to 18 years	3	6.7
18 years and older	39	86.7
All	1	2.2
Sex/gender		
All	45	100.0

**m, month; NP, not provided.*

METHODOLOGICAL QUALITY OF THE INCLUDED CLINICAL TRIALS

Information about clinical trial phase was available in 38 out of 41 interventional studies. Besides, 16 (39.0%) trials belonged to phase 3, 11 (26.8%) to phase 4, and 8 (19.5%) to phase 2. There were 32 (78.1%) trials contained two groups, while six (14.6%) only 1 group. Most trials (78.1%) were randomized. Most commonly adopted intervention model was parallel assignment (n = 32, 78.1%), followed by single group assignment (n = 5, 12.2%). Almost half of the trials (46.4%) were not masked, eight (19.5%) were single masked, and other eight (19.5%) were quadruple masked. Main objectives of the interventional trials lay in treating (61.0%) and preventing (34.2%). Of the four observational studies, two (50.0%) were cohort studies; meanwhile, three (75.0%) trials were prospective and one (25.0%) retrospective (**Table 2**).

DETAILED CHARACTERISTICS OF THE INCLUDED CLINICAL TRIALS

Among the 45 trials, 41 (91.1%) were conducted only on one continent, of which 17 (41.5%) were in North America, 12 (29.3%) in Europe, and six (14.6%) in Asia. Four trials (8.9%) were conducted on two or more continents, and one of them even involved individuals from four continents. Companies were listed as primary sponsors in 18 (40.0%) trials, universities in 11 (24.5%), and hospitals in six

TABLE 2 | Design data of the trials.

Study type		Number	Percent
Interventional (clinical trial)	*Trial phase*		
	Phase 1	2	4.9
	Phase 1/phase 2	1	2.5
	Phase 2	8	19.5
	Phase 3	16	39.0
	Phase 4	11	26.8
	NP	3	7.3
	Number of arms		
	1	6	14.6
	2	32	78.1
	3	1	2.4
	4	2	4.9
	Allocation		
	Non-randomized	3	7.3
	Randomized	32	78.1
	NP	6	14.6
	Intervention model		
	Single group assignment	5	12.2
	Parallel assignment	32	78.1
	Factorial assignment	1	2.4
	Crossover assignment	3	7.3
	Masking (blinding)		
	Open label	19	46.4
	Single	8	19.5
	Double	3	7.3
	Triple	2	4.9
	Quadruple	8	19.5
	NP (provided)	1	2.4
	Primary purpose		
	Treatment	25	61.0
	Prevention	14	34.2
	Health services research	1	2.4
	NP	1	2.4
Observational	*Observational model*		
	Cohort	2	50.0
	Other	1	25.0
	NP	1	25.0
	Time perspective		
	Prospective	3	75.0
	Retrospective	1	25.0

NP, not provided.

TABLE 3 | Detailed characteristics of the included trials.

	Number	Percent
Locations		
Single continent	41	91.1
Asia	6	14.6
Europe	12	29.3
North America	17	41.5
South America	2	4.9
Africa	3	7.3
Oceania	1	2.4
Multiple continents	4	8.9
2 continents	3	75.0
>2 continents	1	25.0
Study sponsor		
University	11	24.5
Hospital	6	13.3
Industry	18	40.0
Other	10	22.2
Collaborators		
NP	32	71.1
Has collaborators	13	28.9
Funder type		
Other	29	64.5
Other/industry	4	8.9
Industry	11	24.4
Industry/U.S. Fed	1	2.2
Data monitoring committee		
Has data monitoring committee	22	48.9
Not have data monitoring committee	15	33.3
NP	8	17.8
Study results		
Has results	6	13.3
No results	39	86.7
Publications of the study		
No publications	34	75.6
Has publications	11	24.4
<2 publications	3	27.3
≥2 publications	8	72.7
IF of publications		
$0 < IF < 5$	3	27.3
$5 \leq IF < 10$	2	18.2
$10 \leq IF < 40$	0	0.0
$IF \geq 40$	6	54.5

NP, not provided.

(13.3%). A small number of trials (28.9%) had collaborations, and one of them had nine collaborations. Most of the trials (64.5%) were supported by other-type funds, followed by industrial funds (24.4%). Less than half of the trials (48.9%) provided DMCs. Only six (13.3%) trials listed results at ClinicalTrials.gov. While 11 (24.4%) trials offered links to webpage publications displaying relevant results, and 10 (22.2%) were linked to PubMed citation through indexed NCT number of the studies. Most of the trials attached publications (72.7%) possessed more than two publications. Among the published trials, eight (72.7%) enjoyed an impact factor (IF) value no less than 5, and 27.3% no less than 40. (**Table 3**).

DESCRIPTION OF DRUGS IN THE INCLUDED CLINICAL TRIALS

Experimental groups in all included trails involved 11 categories and 27 kinds of antibiotics. Of the 41 intervention trials, 37 compared for efficacy across different drugs, and four for different uses of same drugs (oral/intravenous antibiotics). Of the interventional trials, 25 investigated drugs for treatment, 15 for prevention, while one did not provide intervention targets. Among the trials on treatment, experimental group were mostly used to concentrate on antibiotics, three on anti-inflammatory drugs and one on vitamin supplement. Highly interested antibiotics in the trails were glycopeptides ($n = 5$), β-lactams ($n = 5$), and lipopeptides ($n = 5$). Daptomycin and vancomycin were most frequently discussed in experimental group. In control group, antibiotics appeared in all trials except in placebo/conventional treatment. β-Lactam antibiotics appeared most frequently, which included nine kinds of drugs. Vancomycin, gentamycin, and daptomycin were the most common single-drugs in control group. Among the trials on prevention, most experimental groups adopted antibiotics, two

surgical area disinfectants and one probiotic. Most frequently applied antibiotics was glycopeptides (n = 4), and vancomycin represented the most commonly employed single-antibiotic. In control group, antibiotics and surgical area disinfectant were both focused on. Most frequently accepted antibiotics was β-lactams (n = 4), with cefazolin topping the list. In four observational studies, selected drugs mainly were antibiotics and hormones, and most popular antibiotics were quinolones. In addition, most trials (n = 31, 83.8%) evaluated old antibiotics, and six trials (16.2%) assessed new antimicrobials approved by the FDA in recent years (**Table 4**).

DESCRIPTION OF DRUGS IN VARIED TYPES OF CARDIAC-RELATED INFECTIONS

Drugs adopted for the prevention and treatment of cardiac-related infections were mainly concentrated on antibiotics, antivirals, and glucocorticoids. Antivirals were often employed in treating coronary infections, especially valganciclovir. Glycopeptides, β-lactams, and tetracycline antibiotics were often applied to prevent or treat infections related to open heart surgery; peptide antibiotics were for infections associated with

TABLE 4 | Descriptions of drugs in trials.

Study type	Primary purpose	Experimental group			Comparison group		
		Drug type	Drug name	Frequency	Drug type	Drug name	Frequency
Interventional (clinical trial)	Treatment	*Antibiotic drugs*			*Antibiotic drugs*		
		Glycopeptides	Vancomycin	2	β-Lactams	Ceftriaxone	2
			Dalbavancin*	1		Amoxicillin	1
			Oritavancin*	1		Amicillin	1
			Telavancin	1		Penicillin G	1
		β-Lactams	Ceftriaxone	1		Cloxacillin	1
			Benzathine penicillin G	1		Oxacillin	1
			Imipenem	1		Semi-synthetic penicillin	1
			Amoxicillin	1		Synthetic penicillin	1
			Ceftobiprole medocaril*	1		Cefazolin	1
		Tetracycline	Doxycycline	1	Aminoglycosides	Gentamycin	4
		Macrolides	Azithromycin	1		Netilmicin	1
		Antifungals	Fluconazole	1	Glycopeptides	Vancomycin	7
		Quinolones	Levofloxacin	1	Lipopeptides	Daptomycin	3
		Lipopeptides	Daptomycin	5	Other antibiotics	Rifampicin	1
		Aminoglycosides	Gentamicin	1			
		Other antibiotics	Fosfomycin	1			
			CF-301*	1			
			Benznidazole	1			
			Rifabutin	1			
			Rifampicin	1			
		Others	Selenium	1			
			Immunoglobulins	1			
			Colchicine	1			
			Prednisolone	1			
	Prevention	Antibiotic drugs			Antibiotic drugs		
		Glycopeptides	Vancomycin	4	β-Lactams	Cefazolin	2
		β-Lactams	Cefazolin	2		Cephalexin	1
		Tetracycline	D-PLEX®	2		Ceftaroline	1
		Antivirals	Valganciclovir	2	Antivirals	Mycophenolate	1
			Mycophenolate	1	Quinolones	Levofloxacin	1
		Peptides	Polymyxin-B	1	Lincomycin	Clindamycin	1
			Bacitracin	1	Lipopeptides	Daptomycin	1
		Aminoglycosides	Gentamicin	1	Other antibiotics	Linezolid	1
		Other antibiotics	Mupirocin	1	*Others*	Chlorhexidine	1
		Others	Povidone iodine	1			
			Hydrogen peroxide	1			
			Synbiotic 2000	1			
	NP	Lipopeptides	Daptomycin	1			
Observational	Treatment	Antibiotic drugs					
		Quinolones	Trovafloxacin	1			
			Levofloxacin	1			
		Lipopeptides	CUBICIN	1			
		Other antibiotics	Fosfomycin	1			
		Others	Hydrocortisone	1			

**U.S. FDA-regulated Drug Product; NP, not provided.*

implantations surgery. Lipopeptides were most commonly studied, followed by β-lactams and glycopeptides among the IE caused by *Staphylococci*. Glycopeptides, especially vancomycin, most commonly appeared in methicillin-resistant *Staphylococcus aureus* (MRSA)-induced IE studies, while β-lactams in those on IE caused by *Enterococcus faecalis*. Glucocorticoids were most adopted in heart diseases that cause systemic infections. In addition, there were also other non-antibiotics involved. In these studies, especially those related to IE, three drugs were adopted to explore effects of different modes of administration (oral *vs* intravenous treatment), and one drug for pharmacokinetic characteristics of different doses and frequency (**Table 5**).

DISCUSSION

This study comprehensively analyzed drug trials registered on ClinicalTrials.gov, all of which explored the intervention of infectious heart diseases or cardiac-related surgery infections. Through the analysis, we found that the number of registered cardiac-related infectious diseases was less than that of infectious diseases related to other organs. In these trials, most took interventional design. One-third of the trials were completed and 15.6% terminated. Most intervention trials were in phase 3 or 4, randomized, parallel assignment, masking, and sufficient, and possessed large sample size. Meanwhile, 24.4% of the trials offered publication links accessing to study results, 13.3% uploaded their results, and less than half provided DMCs. Drugs for treatment were mainly antibiotics, with glycopeptides, β-lactams, and lipopeptides topping the list in experimental group, while glycopeptides dominated in experimental group among trails on prevention.

From the perspective of study design, the vast majority of the trials (78.1%) were randomized and parallel assignment with two arms. Randomization, an exceptionally powerful tool, largely prevents confusion and mitigates selection bias in treatment comparisons (Sessler and Imrey, 2015). Besides, 51.2% of the trials were blinded. Well-implemented masking simultaneously prevents measurement bias and placebo effects through balancing treatment effects (Devereaux and Yusuf, 2003). Meanwhile, 62.2% of the trials contained more than 100 participants, and 20.0% more than 1,000. Sample size affects many factors, like statistical power, effect size, population mean, and variance (Allareddy et al., 2014). Due to the lack of information on the ClinicalTrials.gov Registry, we failed to make accurate judgments. However, larger sample size can increase the accuracy of estimated treatment outcome in trail and results' credibility (Ruberg and Akacha, 2017). The standardization of clinical study design is important in successfully implementing clinical research. Appropriate randomization method, adequate masking, and treatment assignment, selecting active comparator and reasonable target sample size are essential in realizing reliable (unbiased) treatment comparison (Pocock et al., 2015). But many trials did not mention specific procedures for randomization, allocation concealment, or the phase of open label. Therefore, we could not determine whether they possessed high quality. Additionally, 37.8% of the trials spanned more than 36 months, and 11.1% even lasted beyond 72 months. As for study time prolonging, time-dependent bacterial resistance rate would become a significant confounding factor (Wan et al., 2018). Because of varied study duration periods, bacterial resistance rates were different. When comparing drug efficacy, this aspect would possibly bias research conclusion (Venekamp et al., 2016). However, most trials chose random enrollment to alleviate the impact of this aspect. Of the trials, 17.8% were terminated or withdrawn, mainly due to inadequate enrollment, followed by lacking statistical power and business reasons. In addition, only 13.3% of the trials included children. The shortage of funds for children's medication and insufficient drug development still represent major challenges facing clinical studies on children's medications (Allegaert et al., 2018). We hope that the government can introduce relevant policies to encourage more research institutions to conduct drug trials on children to establish optimal clinical treatment strategies.

The selected trails were implemented in six continents, and 8.9% of them were conducted in two or more continents. As shown on the official website, ClinicalTrials.gov is a database of global clinical studies receiving both private and public funds. It covers a wide geographical range, making us accessible to more comprehensive information on diseases and more reliable results. Of the eligible trails, 28.9% had collaborators. Large-scale multicenter trials, exceeding single-center ones, would facilitate the recruitment of enough patients, speed trials' progress (Brophy, 2015), and improve research's external validity (Allareddy et al., 2014). However, to reduce the bias in study implementation, it is necessary for researchers in different institutions to accept uniform standards and to reach consistent understanding (American Society of Clinical Oncology, 2003). Besides, 48.9% of the trials offered DMCs; while DMC is vital in maintaining scientific integrity in trials, the authenticity and accuracy of trial data, and the safety of studied participants (Filippatos et al., 2017). Only 13.3% of the trials showed results on ClinicalTrials.gov, partly due to the presence of various extensions and exemptions. The Food and Drug Administration Amendments Act of 2007 (FDAAA) demands to submit "basic results" for certain types of clinical trials within 1 year after experiment completion (Phillips et al., 2017). Reporting study results is critical in advancing study progression and ensuring the safety of participants in clinical trials. Therefore, it is necessary to strengthen the supervision of online result publication (Lee et al., 2018). Among our selected trails, 24.4% offered web links to their publications with relevant results on ClinicalTrials.gov., of which 27.3% were published on top journals (IF ≥ 40). Good research design plays a crucial role in result publication (del Rio et al., 2014). At the same time, we found that most of the trials (66.7%) on top journals were funded by universities, hospitals, and research institutions, and industry-funded ones accounted for only one-third. Recent evidence indicates that trials funded by industry sources are likely to be biased in favor of sponsors' products, thus causing obvious publication bias (Lundh et al., 2017).

In general, the vast majority of the trials focused on treating or preventing the occurrence of infection adopting antibiotics. Six trials (16.2%) evaluated new antimicrobials approved by the

TABLE 5 | Description of drugs in varied types of cardiac-related infections.

Conditions/diseases		Experimental group			Comparison group		
		Antibiotic drugs	Drug name	F	Antibiotic drugs	Drug name	F
Coronary infection	Coronary heart disease/ Chlamydophila pneumoniae infections	Tetracycline	Doxycycline	1			
		Macrolides	Azithromycin	1			
		Others	Rifabutin	1			
	Cardiac allograft vasculopathy/ Cytomegalovirus infection	Antivirals	Valganciclovir	3	Antivirals	Pre-emptive mycophenolate	1
			Mycophenolate	1			
Cardiac surgery infection	Cardiac surgery/SSIs	Glycopeptides	Vancomycin	3	β-Lactams	Cefazolin	2
		β-Lactams	Cefazolin	2			
		Tetracycline	D-PLEX*	2			
		Aminoglycosides	Gentamicin	1			
		Antivirals	Valganciclovir	1			
		Others	Mupirocin	1			
		Non-antibiotic	Synbiotic 2000	1			
	Implantations surgery/infections	Non-antibiotic	Povidone iodine	1	Non-antibiotic	Chlorhexidine	1
			Hydrogen peroxide	1	β-Lactams	Cephalexin	1
		Others	Fluconazole	1	Linkes	Clindamycin	1
		Peptide antibiotics	Polymyxin B/ bacitracin	1	Peptide antibiotics	Polymyxin B/bacitracin	1
					Quinolones	Levofloxacin	1
Infective endocarditis (IE)	IE/bacterial	Others	Fosfomycin	1			
	IE/microbial infection	Glycopeptides	Vancomycin	1	Aminoglycosides	Gentamycin	1
	IE/*Streptococci*, *Staphylococci* or *Enterococci* infecting		Antibiotic therapy#				
	IE/*Streptococcus-Enterococcus*	β-Lactams	Amoxicillin#	1	β-Lactams	Amoxicillin	1
						Ampicillin	1
						Penicillin G	1
						Ceftriaxone	1
					Aminoglycosides	Vancomycin	1
						Gentamicin	1
						Netilmicin	1
	IE/MRSA, *Streptococci*	Glycopeptides	Dalbavancin*	1			
	IE/MRSA	Glycopeptides	Vancomycin	1	Glycopeptides	Vancomycin	2
		Others	Fosfomycin	1			
		β-Lactams	Imipenem	1			
		Lipopeptides	Daptomycin	1			
	IE/SA	Lipopeptides	CUBICIN	1	Lipopeptides	Daptomycin	4
			Daptomycin	4			
		Quinolones	Levofloxacin#	2	Glycopeptides	Vancomycin	4
			Trovafloxacin	1	β-Lactams	Cloxacillin,	1
		Glycopeptides	Telavancin	1		Oxacillin,	1
			Vancomycin	1		Semi-synthetic Penicillin	1
						Synthetic penicillin	1
		β-Lactams	Ceftobiprole medocaril*	1		Cefazolin	1
		Aminoglycosides	Gentamici	1		Ceftaroline	1
		Others	CF-301*	1	Aminoglycosides	Gentamicine	2
			Rifampicin,1#	1	Others	Rifampicin	1
						Linezolid	1
	IE/*Enterococcus faecalis*	β-Lactams	Ceftriaxone@	1	β-Lactams	Ceftriaxone	1
		Lipopeptides	Daptomycin	1			
	IE/OUD	Glycopeptides	Oritavancin injection*	1			
			OPAT#	1			
Infectious myocarditis	Chagasic myocardiopathy/ *Trypanosoma cruzi*	Others	Benznidazole	1			
		Non-antibiotic	Selenium	1			
			Colchicine	1			
	Myocardial diseases/ Parvovirus B19	Non-antibiotic	Intravenous Immunoglobulins	1			
Infectious valvulitis	RHD/group A *Streptococcus*	β-Lactams	Benzathine penicillin G	1			

TABLE 5 | Continued

Conditions/diseases		Experimental group			Comparison group		
		Antibiotic drugs	Drug name	F	Antibiotic drugs	Drug name	F
Infectious pericarditis	Tuberculous pericarditis/HIV	Non-antibiotic	Prednisolone	1			
CA/infection	Post-resuscitation infection/ infections	Non-antibiotic	Hydrocortisone	1			

**U.S. FDA-regulated Drug Product; #the oral treatment vs IV treatment; @Different doses of the same drug; F, frequency; SSIs, surgical site infections; MRSA, methicillin-resistant Staphylococcus aureus; SA, Staphylococcus aureus bacteria; OPAT, outpatient parenteral antibiotic therapy; OUD, opioid use disorder; RHD, rheumatic heart disease; HIV, human immunodeficiency virus; CA, cardiac arrest.*

FDA in recent years. Since many pathogens are more resistant to existing antibiotics, it has become particularly important to develop new antibiotics to improve time-related bacterial resistance rates (Penchovsky and Traykovska, 2015). Two trials evaluated the efficacy and safety of different methods of using the same antibiotic. Given the global crisis of antimicrobial resistance, it is important to determine the optimal duration of applying intravenous and oral antibiotics and to form evidence-based recommendations about when to switch from intravenous to oral routes (McMullan et al., 2016).

Diseases in the selected trials involved infective endocarditis (IE), Chagas heart disease, coronary infection, parvovirus-mediated cardiomyopathy, tuberculous pericarditis, childhood rheumatic heart disease (RHD), and heart-related device/surgery infections. Options are limited for treating endocarditis caused by MRSA. Vancomycin is the standard treatment for blood infections caused by MRSA, but its effect is limited in treating endocarditis caused by MRSA. Its bactericidal activity is weaker than that of β-lactams, showing low permeability in the valves, while debates still exist on its applicability (del Rio et al., 2014). Many clinical trials have evaluated the efficiency of different types or doses of antibiotic treatments, such as daptomycin, CF-301, β-lactams, fosfomycin, dalbavancin, levofloxacin, and new glycopeptides (dalbavancin or oritavancin). Earlier researches also involved optimized antibiotics treatment options for IE patients with opioid use disorder (OUD), an infection that has recently doubled hospitalization rates. In addition, the effectiveness and safety of conversion from intravenous antibiotics to oral ones have also been well evaluated. The effect of Trypanosoma treatment on Chagas myocardiopathy caused by *T. cruzi* infection is still unclear. Benznidazole, colchicine, and selenium supplementation were included in the study to explore their clinical efficacy. Besides, two of our enrolled trials examined the effects of combined antibiotics or antiviral drugs on preventing changes in coronary vascular infections. In addition, an included trial investigated the effect of high-dose intravenous immunoglobulin on virus presence in patients with high load of parvovirus B19 in the heart. Tuberculous pericarditis is often accompanied by human immunodeficiency virus (HIV) infection, seeing poor prognosis, and a collected trial evaluated the efficacy and safety of adjunctive prednisolone therapy. Appropriate management for latent RHD has not been developed and no formal recommendations have been established. Some trials explored the prophylaxis and prognosis of intramuscular benzathine penicillin G (BPG). Despite the use of prophylactic systemic antibiotics, postoperative wound infections and infections associated with cardiac implantable electronic devices still represent serious threats after heart-related surgery (Mertz et al., 2011; Arnold and Chu, 2018), though many types of antibiotic treatment regimens have been compared and comprehensively analyzed. For example, when it comes to D-PLEX, vancomycin, mupirocin, etc., whether their applications should be directly intravenous, oral, topical, or through dressing during surgery has been constantly discussed.

According to published articles, patients with IE who received daptomycin treatment could reach similar outcomes to those treated with vancomycin, and such treatment could be used as a reasonable alternative (Fowler et al., 2006). The combination of fosfomycin with imipenem prevents drug resistance caused by single drug, and small doses can achieve ideal efficacy with reduced side effects among IE patients (del Rio et al., 2014). Fluoroquinolone combined with standard treatment does not improve treatment outcomes of *S. aureus bacteremia*, nor does it reduce mortality or the incidence of deep infection (Ruotsalainen et al., 2006). In patients with stable clinical conditions and sufficient response to initial treatment, transformation from intravenous administration to oral antibiotic treatment is not inferior to continued intravenous antibiotic treatment. Oral antibiotic may also minimize problems associated with outpatient parenteral treatment, logistics, and monitoring, and the risks of complications associated with intravenous catheters (e.g., local and systemic infections, and venous thrombosis) (Iversen et al., 2019). In a large randomized controlled trial, benznidazole significantly reduced the detection rate of parasitic infections in Chagas heart disease patients but did not significantly reduce the incidence of major clinical outcomes (Morillo et al., 2015). Adjunctive prednisolone had no significant effect on major clinical outcomes (Mayosi et al., 2014). Gentamicin-impregnated dressing had no significant effect on the rate of wound infection in patients undergoing open cardiac surgery (Bennett-Guerrero et al., 2010). Consequently, specific improvements in antibiotics, including the use of narrow-spectrum therapy, shortening treatment times, early transition from IV to oral therapy (Barlam et al., 2016), and the development of new drugs to prevent and treat infections, are key strategies in combating antimicrobial resistance.

The process of preventing and treating infectious diseases is complicated. Choosing right medication regimen, especially antibiotic regimen, requires a combination of the characteristics of

pathogenic microorganism, the duration of medication application, adverse events caused by medication, and other outcomes (Cahill and Prendergast, 2016). For example, IE, one of the most common diseases associated with heart-related infectious diseases, is hard to be cured due to the characteristics of the infection itself, the bacterial species, and frequent comorbidities of the patients (del Rio et al., 2014; Schirone et al., 2018). A long treatment cycle (4–6 weeks of intravenously administered antibiotic agents) used to be required, and aminoglycoside often brings about side effect of nephrotoxicity (Baddour et al., 2015). In addition, the rate of antibiotic-resistant strains is increasing, which makes the establishment of effective antibiotic regimes more complicated. For this reason, some alternatives have been explored: Native valve endocarditis (NVE) was often treated with penicillin G and gentamicin for synergistic coverage of *Streptococci*. Patients with a history of intravenous drug use were treated with nafcillin and gentamicin to cover for methicillin-sensitive *Staphylococci*. The emergence of MRSA and penicillin-resistant *Streptococci* led to changes in empiric treatment, with liberal substitution of vancomycin in lieu of a penicillin antibiotic (Razmi and Magnusson, 2019). Treatment options range from in-hospital intravenous antibiotic therapy to partial oral therapy, replacement has minimized challenges associated with outpatient parental treatment (Boucher, 2019). Treatment offers the possibility of benefiting more patients, with multidisciplinary collaborative approach for the management of IE (Tan et al., 2018).

A recent review published in *Nature Reviews Immunology* (Parihar et al., 2019) presented a unique perspective on new drugs for the intervention of infectious diseases. As a host-directed treatment, statins wield powerful effects against infectious diseases caused by viruses, parasites, fungi, and bacteria. In particular, statin-mediated destructive effects, in combination with standard therapies, could interfere with microdomain lipids of MRSA, thus providing a novel anti-multidrug resistance infection strategy (Thangamani et al., 2015). However, we still need proof-of-concept clinical studies and large randomized controlled trials to verify the feasibility of statins acting as potential replacement therapy in infectious diseases.

Our research still had some limitations: 1) Clinical trials were obtained only from ClinicalTrials.gov, though this source contains most of global trials; and we might miss some trials registered in other 11 registries (Zarin et al., 2011) that were not fully evaluated. 2) Regarding to trial search and data extraction performed in this study, although all words supporting extensive research were used to maximize the number of included trials, some studies related to cardiac infection may not be found. 3) Since all information was obtained from ClinicalTrials.gov, intelligence not in this source was not further evaluated. At the same time, we only interpreted results acquired through ClinicalTrials.gov online links, and no more relevant publications were manually retrieved in the National Center for Biotechnology Information (NCBI) database using registration numbers. 4) Although final conclusions were relatively reliable based on publications with online links, considering that most of them were published in high-scoring journals, final establishment of clinical treatment methods need to take clinical therapies into account, after referring to more high-quality articles.

CONCLUSIONS

Our study comprehensively analyzes the characteristics of trials registered at ClinicalTrials.gov for drug prevention or treatment of cardiac-related infections. Most clinical trials were interventional RCTs for treatment. While the majority of interested drugs were old antibiotics, and few trials reported valid study results. It is necessary to strengthen supervision over the improvement of trial results, and to implement explorations combining antibacterial activity with drug delivery regimens to achieve optimal clinical outcomes.

AUTHOR CONTRIBUTIONS

X-CL designed this study. L-LM and YQ performed the search and collected data, M-NS re-checked data. YC and J-XQ assessed and analyzed the data, and B-HL and M-JZ re-checked, assessed, and analyzed, L-LM and M-NS wrote the manuscript, X-CL reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00757/full#supplementary-material

REFERENCES

Allareddy, V., Rampa, S., Masoud, M. I., Lee, M. K., Nalliah, R., and Allareddy, V. (2014). Overview of registered studies in orthodontics: evaluation of the ClinicalTrials.gov registry. *Am. J. Orthod. Dentofacial. Orthop.* 146 (5), 587–593. doi: 10.1016/j.ajodo.2014.07.019

Allegaert, K., Smits, A., and van den Anker, J. N. (2018). Drug evaluation studies in neonates: how to overcome the current limitations. *Expert Rev. Clin. Pharmacol.* 11 (4), 387–396. doi: 10.1080/17512433.2018.1439378

American Society of Clinical Oncology (2003). American Society of Clinical Oncology policy statement: oversight of clinical research. *J. Clin. Oncol.* 21 (12), 2377–2386. doi: 10.1200/JCO.2003.04.026

Arnold, C. J., and Chu, V. H. (2018). Cardiovascular implantable electronic device infections. *Infect. Dis. Clin. North Am.* 32 (4), 811–825. doi: 10.1016/j.idc.2018.06.004

Baddour, L. M., Wilson, W. R., Bayer, A. S., Fowler, V. G., Jr., Tleyjeh, I. M., Rybak, M. J., et al. (2015). Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare

professionals from the american heart association. *Circulation* 132 (15), 1435–1486. doi: 10.1161/CIR.0000000000000296

Barlam, T. F., Cosgrove, S. E., Abbo, L. M., MacDougall, C., Schuetz, A. N., Septimus, E. J., et al. (2016). Implementing an antibiotic stewardship program: guidelines by the infectious diseases society of America and the society for healthcare epidemiology of America. *Clin. Infect. Dis.* 62 (10), e51–77. doi: 10.1093/cid/ciw118

Bennett-Guerrero, E., Ferguson, T. B., Jr., Lin, M., Garg, J., Mark, D. B., Scavo, V. A., Jr., et al. (2010). Effect of an implantable gentamicin-collagen sponge on sternal wound infections following cardiac surgery: a randomized trial. *JAMA* 304 (7), 755–762. doi: 10.1001/jama.2010.1152

Boucher, H. W. (2019). Partial oral therapy for osteomyelitis and endocarditis - is it time? *N. Engl. J. Med.* 380 (5), 487–489. doi: 10.1056/NEJMe1817264

Brophy, J. M. (2015). Multicenter trials, guidelines, and uncertainties - Do we know as much as we think we do? *Int. J. Cardiol.* 187, 600–603. doi: 10.1016/j.ijcard.2015.04.004

Burnham, J. P., Olsen, M. A., and Kollef, M. H. (2019). Re-estimating annual deaths due to multidrug-resistant organism infections. *Infect. Control Hosp. Epidemiol.* 40 (1), 112–113. doi: 10.1017/ice.2018.304

Cahill, T. J., and Prendergast, B. D. (2016). Infective endocarditis. *Lancet* 387 (10021), 882–893. doi: 10.1016/S0140-6736(15)00067-7

Califf, R. M., Zarin, D. A., Kramer, J. M., Sherman, R. E., Aberle, L. H., and Tasneem, A. (2012). Characteristics of clinical trials registered in ClinicalTrials.gov, 2007-2010. *JAMA* 307 (17), 1838–1847. doi: 10.1001/jama.2012.3424

Chirouze, C., Alla, F., Fowler, V. G., Jr., Sexton, D. J., Corey, G. R., Chu, V. H., et al. (2015). Impact of early valve surgery on outcome of Staphylococcus aureus prosthetic valve infective endocarditis: analysis in the International Collaboration of Endocarditis-Prospective Cohort Study. *Clin. Infect. Dis.* 60 (5), 741–749. doi: 10.1093/cid/ciu871

Cihoric, N., Tsikkinis, A., Minniti, G., Lagerwaard, F. J., Herrlinger, U., Mathier, E., et al. (2017). Current status and perspectives of interventional clinical trials for glioblastoma - analysis of ClinicalTrials.gov. *Radiat. Oncol.* 12 (1), 1. doi: 10.1186/s13014-016-0740-5

De Angelis, C. D., Drazen, J. M., Frizelle, F. A., Haug, C., Hoey, J., Horton, R., et al. (2005). Is this clinical trial fully registered?–A statement from the International Committee of Medical Journal Editors. *N. Engl. J. Med.* 352 (23), 2436–2438. doi: 10.1056/NEJMe058127

del Rio, A., Gasch, O., Moreno, A., Pena, C., Cuquet, J., Soy, D., et al. (2014). Efficacy and safety of fosfomycin plus imipenem as rescue therapy for complicated bacteremia and endocarditis due to methicillin-resistant Staphylococcus aureus: a multicenter clinical trial. *Clin. Infect. Dis.* 59 (8), 1105–1112. doi: 10.1093/cid/ciu580

Devereaux, P. J., and Yusuf, S. (2003). The evolution of the randomized controlled trial and its role in evidence-based decision making. *J. Intern. Med.* 254 (2), 105–113. doi: 10.1046/j.1365-2796.2003.01201.x

Filippatos, G. S., de Graeff, P., Bax, J. J., Borg, J. J., Cleland, J. G., Dargie, H. J., et al. (2017). Independent academic Data Monitoring Committees for clinical trials in cardiovascular and cardiometabolic diseases. *Eur. J. Heart Fail* 19 (4), 449–456. doi: 10.1002/ejhf.761

Fong, I. W. (2009). New perspectives of infections in cardiovascular disease. *Curr. Cardiol. Rev.* 5 (2), 87–104. doi: 10.2174/157340309788166679

Fowler, V. G., Jr., Boucher, H. W., Corey, G. R., Abrutyn, E., Karchmer, A. W., Rupp, M. E., et al. (2006). Daptomycin versus standard therapy for bacteremia and endocarditis caused by Staphylococcus aureus. *N. Engl. J. Med.* 355 (7), 653–665. doi: 10.1056/NEJMoa053783

Friedrich, A. W. (2019). Control of hospital acquired infections and antimicrobial resistance in Europe: the way to go. *Wien. Med. Wochenschr.* 169 (Suppl 1), 25–30. doi: 10.1007/s10354-018-0676-5

Habib, G., Lancellotti, P., Antunes, M. J., Bongiorni, M. G., Casalta, J. P., Del Zotti, F., et al. (2015). 2015 ESC Guidelines for the management of infective endocarditis: The Task Force for the Management of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by: European Association for cardio-thoracic surgery (EACTS), the European association of nuclear medicine (EANM). *Eur. Heart J.* 36 (44), 3075–3128. doi: 10.1093/eurheartj/ehv319

Hughes, D. (2014). Selection and evolution of resistance to antimicrobial drugs. *IUBMB Life* 66 (8), 521–529. doi: 10.1002/iub.1278

Iversen, K., Ihlemann, N., Gill, S. U., Madsen, T., Elming, H., Jensen, K. T., et al. (2019). Partial oral versus intravenous antibiotic treatment of endocarditis. *N. Engl. J. Med.* 380 (5), 415–424. doi: 10.1056/NEJMoa1808312

Lee, T. E., Kim, A., Jang, M., and Jeon, B. (2018). Underregistration and Underreporting of Stem Cell Clinical Trials in Neurological Disorders. *J. Clin. Neurol.* 14 (2), 215–224. doi: 10.3988/jcn.2018.14.2.215

Lundh, A., Lexchin, J., Mintzes, B., Schroll, J. B., and Bero, L. (2017). Industry sponsorship and research outcome. *Cochrane Database Syst. Rev.* 2, Mr000033. doi: 10.1002/14651858.MR000033.pub3

Mayosi, B. M., Ntsekhe, M., Bosch, J., Pandie, S., Jung, H., Gumedze, F., et al. (2014). Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. *N. Engl. J. Med.* 371 (12), 1121–1130. doi: 10.1056/NEJMoa1407380

McMullan, B. J., Andresen, D., Blyth, C. C., Avent, M. L., Bowen, A. C., Britton, P. N., et al. (2016). Antibiotic duration and timing of the switch from intravenous to oral route for bacterial infections in children: systematic review and guidelines. *Lancet Infect. Dis.* 16 (8), e139–152. doi: 10.1016/S1473-3099(16)30024-X

Mentzelopoulos, S. D., Malachias, S., Chamos, C., Konstantopoulos, D., Ntaidou, T., Papastylianou, A., et al. (2013). Vasopressin, steroids, and epinephrine and neurologically favorable survival after in-hospital cardiac arrest: a randomized clinical trial. *JAMA* 310 (3), 270–279. doi: 10.1001/jama.2013.7832

Mertz, D., Johnstone, J., and Loeb, M. (2011). Does duration of perioperative antibiotic prophylaxis matter in cardiac surgery? A systematic review and meta-analysis. *Ann. Surg.* 254 (1), 48–54. doi: 10.1097/SLA.0b013e318214b7e4

Morillo, C. A., Marin-Neto, J. A., Avezum, A., Sosa-Estani, S., Rassi, A., Jr., Rosas, F., et al. (2015). Randomized trial of benznidazole for chronic chagas cardiomyopathy. *N. Engl. J. Med.* 373 (14), 1295–1306. doi: 10.1056/NEJMoa1507574

Nadji, G., Remadi, J. P., Coviaux, F., Mirode, A. A., Brahim, A., Enriquez-Sarano, M., et al. (2005). Comparison of clinical and morphological characteristics of Staphylococcus aureus endocarditis with endocarditis caused by other pathogens. *Heart* 91 (7), 932–937. doi: 10.1136/hrt.2004.042648

Parihar, S. P., Guler, R., and Brombacher, F. (2019). Statins: a viable candidate for host-directed therapy against infectious diseases. *Nat. Rev. Immunol.* 19 (2), 104–117. doi: 10.1038/s41577-018-0094-3

Penchovsky, R., and Traykovska, M. (2015). Designing drugs that overcome antibacterial resistance: where do we stand and what should we do? *Expert Opin. Drug Discov* 10 (6), 631–650. doi: 10.1517/17460441.2015.1048219

Phillips, A. T., Desai, N. R., Krumholz, H. M., Zou, C. X., Miller, J. E., and Ross, J. S. (2017). Association of the FDA Amendment Act with trial registration, publication, and outcome reporting. *Trials* 18 (1), 333. doi: 10.1186/s13063-017-2068-3

Pocock, S. J., Clayton, T. C., and Stone, G. W. (2015). Design of major randomized trials: Part 3 of a 4-part series on statistics for clinical trials. *J. Am. Coll. Cardiol.* 66 (24), 2757–2766. doi: 10.1016/j.jacc.2015.10.036

Razmi, R., and Magnusson, P. (2019). [Management of infective endocarditis]. *Lakartidningen* 116. FEPZ.

Ruberg, S. J., and Akacha, M. (2017). Considerations for Evaluating Treatment Effects From Randomized Clinical Trials. *Clin. Pharmacol. Ther.* 102 (6), 917–923. doi: 10.1002/cpt.869

Ruotsalainen, E., Jarvinen, A., Koivula, I., Kauma, H., Rintala, E., Lumio, J., et al. (2006). Levofloxacin does not decrease mortality in Staphylococcus aureus bacteraemia when added to the standard treatment: a prospective and randomized clinical trial of 381 patients. *J. Intern. Med.* 259 (2), 179–190. doi: 10.1111/j.1365-2796.2005.01598.x

Schirone, L., Iaccarino, A., Saade, W., D'Abramo, M., De Bellis, A., Frati, G., et al. (2018). Cerebrovascular complications and infective endocarditis: impact of available evidence on clinical outcome. *Biomed. Res. Int.* 2018, 4109358. doi: 10.1155/2018/4109358

Sessler, D. I., and Imrey, P. B. (2015). Clinical Research Methodology 3: Randomized controlled trials. *Anesth. Analg.* 121 (4), 1052–1064. doi: 10.1213/ANE.0000000000000862

Tan, C., Hansen, M. S., Cohen, G., Boyle, K., Yang, A., Rishu, A., et al. (2018). Case conferences for infective endocarditis: a quality improvement initiative. *PLoS One* 13 (10), e0205528. doi: 10.1371/journal.pone.0205528

Thangamani, S., Mohammad, H., Abushahba, M. F., Hamed, M. I., Sobreira, T. J., Hedrick, V. E., et al. (2015). Exploring simvastatin, an antihyperlipidemic drug, as a potential topical antibacterial agent. *Sci. Rep.* 5, 16407. doi: 10.1038/srep16407

Thornhill, M. H., Gibson, T. B., Cutler, E., Dayer, M. J., Chu, V. H., Lockhart, P. B., et al. (2018). Antibiotic prophylaxis and incidence of endocarditis before and after the 2007 AHA recommendations. *J. Am. Coll. Cardiol.* 72 (20), 2443–2454. doi: 10.1016/j.jacc.2018.08.2178

Venekamp, R. P., Burton, M. J., van Dongen, T. M., van der Heijden, G. J., van Zon, A., and Schilder, A. G. (2016). Antibiotics for otitis media with effusion in children. *Cochrane Database Syst. Rev.* (6), Cd009163. doi: 10.1002/14651858.CD009163.pub3

Wan, H., Yi, X., Liu, X., Feng, C., Dang, Z., and Wei, C. (2018). Time-dependent bacterial community and electrochemical characterizations of cathodic biofilms in the surfactant-amended sediment-based bioelectrochemical reactor with enhanced 2,3,4,5-tetrachlorobiphenyl dechlorination. *Environ. Pollut.* 236, 343–354. doi: 10.1016/j.envpol.2018.01.048

Zarin, D. A., Tse, T., Williams, R. J., Califf, R. M., and Ide, N. C. (2011). The ClinicalTrials.gov results database–update and key issues. *N. Engl. J. Med.* 364 (9), 852–860. doi: 10.1056/NEJMsa1012065

Zumla, A., Rao, M., Wallis, R. S., Kaufmann, S. H., Rustomjee, R., Mwaba, P., et al. (2016). Host-directed therapies for infectious diseases: current status, recent progress, and future prospects. *Lancet Infect. Dis.* 16 (4), e47–63. doi: 10.1016/S1473-3099(16)00078-5

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Immunological Efficacy of Tenofovir Disproxil Fumarate-Containing Regimens in Patients With HIV-HBV Coinfection: A Systematic Review and Meta-Analysis

Taiyi Jiang[1,2†], Bin Su[1,2†], Ting Song[1,2], Zhiqiang Zhu[3], Wei Xia[1,2], Lili Dai[1], Wen Wang[1], Tong Zhang[1,2] and Hao Wu[1,2*]*

[1] Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, China, [2] Beijing Key Laboratory for HIV/AIDS Research, Beijing, China, [3] Department of Urology, Beijing Youan Hospital, Capital Medical University, Beijing, China

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Edited by:
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University of Science, Malaysia

***Correspondence:**
Tong Zhang
zt_doc@ccmu.edu.cn
Hao Wu
whdoc@ccmu.edu.cn

†These authors have contributed equally to this work

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Background: Hepatitis B virus (HBV) coinfection is common in HIV-positive patients. HIV infection modifies the natural course of HBV infection, leading to a faster progression of liver-related morbidity and mortality than is observed in HBV mono-infected patients. This systematic review and meta-analysis evaluates the current clinical evidence regarding the use of oral tenofovir disproxil fumarate (TDF)-based treatments in patients coinfected with HIV and HBV.

Methods: We performed a comprehensive literature search in PubMed and Web of Science. Supplementary searches were conducted in Google Scholar and Clinicaltrials.gov. We conducted a random effects meta-analysis using the event rate (ER) to estimate the incidence of HBV seroconversion. A subgroup meta-analysis was performed to assess the moderate effects of demographic and disease-related variables on HBsAg loss. This review is registered in the PROSPERO database (CRD42018092379).

Results: We included 11 studies in the review. The immunological effects of oral TDF-based Pre-exposure prophylaxis (PrEP) treatment in patients with HIV-HBV coinfection were 0.249 for HBeAg loss, 0.237 for HBeAg conversion, 0.073 for HBsAg loss, and 0.055 for HBsAg conversion. The factors associated with HBsAg loss were the baseline HBV viral load, participant's location, and a history of exposure to lamivudine/emtricitabine (3TC/FTC) (all $p < 0.05$). A trend toward a negative relationship between the baseline $CD4^+$ T-cell count and HBsAg loss was observed ($p = 0.078$).

Conclusion: This systematic review and meta-analysis demonstrated that TDF-containing regimens are effective at stimulating HBeAg loss (24.9%), HBeAg conversion (23.7%), HBsAg loss (7.3%), and HBsAg conversion (5.5%) in HIV-HBV coinfected patients. The moderator analysis showed that HBV viral load, the location of participants, and prior exposure to 3TC/FTC are factors associated with HBsAg loss. Asian ethnicity, prior exposure to 3TC, and a nondetectable baseline HBV viral load are associated with lower odds of HBsAg loss. Well-designed prospective cohort studies and randomized controlled trials (RCTs) with large sample sizes are required for the investigation of potential

predictors and biological markers associated with strategies for achieving HBV remission in patients with HIV-HBV coinfection, which is a matter of considerable importance to clinicians and those responsible for health policies.

Keywords: tenofovir disproxil fumarate, drug treatment, outcomes research, meta-analysis, HIV, hepatitis B virus, coinfection

INTRODUCTION

Approximately 5–25% of acquired immunodeficiency syndrome (AIDS) patients are coinfected with hepatitis B virus (HBV) (Unaids, 2018). Human immunodeficiency virus (HIV) infection modifies the natural course of HBV infection, leading to a faster progression of liver-related morbidity and mortality than is observed in HBV mono-infected individuals, accompanied by a higher prevalence of antiretroviral therapy (ART)-related hepatotoxicity (Avihingsanon et al., 2010). Recent studies have reported that liver disease continues to progress in 10–20% of individuals on tenofovir-containing HBV-active ART (Coffin et al., 2013; Vinikoor et al., 2017). Tenofovir disproxil fumarate (TDF) is one of the most commonly/widely used nucleotide reverse transcriptase inhibitors (NRTI) for the treatment of HIV and HBV and is recommended by the current HIV treatment guidelines (Aidsinfo, 2018). TDF-containing regimens are particularly favored for the clinical treatment of HIV-HBV coinfection in areas in which resources are limited.

Tenofovir is widely used as a first-line agent for the treatment of chronic HBV infection due to the relatively low levels of drug resistance and high virological efficacy (Nunez et al., 2002; Ristig et al., 2002; Dore et al., 2004; Bihl et al., 2015). A recent study showed that TDF treatment resulted in undetectable levels of HBV in approximately 90% of patients with HIV-HBV coinfection. This proportion increased rapidly over the first 2 years of treatment and continued to rise slowly thereafter (Price et al., 2013). Moreover, there is currently no confirmed evidence of mutations conferring resistance to TDF in the HBV strains harbored by these patients (Kitrinos et al., 2014).

Interest has recently focused on trying to cure chronic HBV infection. NRTI treatment has been shown to decrease the formation of stable episomal covalently closed circular DNA (cccDNA) and, to a lesser extent, the integration of HBV DNA into the host genome, but HBsAg continues to be produced. Sustained high levels of HBsAg have been associated with a high risk of hepatocellular carcinoma (HCC) in cases of untreated HBV mono-infection (Tseng et al., 2012; Yang et al., 2016). The persistence of cccDNA and HBsAg are the main barriers to curing HBV (Zeisel et al., 2015). HBsAg seroclearance and the development of antibodies against HBsAg can be used to assess HBV function.

Prolonged periods of good response to TDF treatment have been achieved, with HBV DNA remaining undetectable in the serum, but the elimination of cccDNA is the ultimate goal in strategies that aim to cure HBV infection. The concept of a functional cure, defined as the clearance of HBsAg or persistent seroconversion during treatment that may also improve clinical outcomes, has recently been proposed. A loss of the HBsAg biomarker and seroconversion are clearly associated with lower levels of viral activity in the liver and the achievement of HBV remission. The hepatitis B "e" antigen (HBeAg) can also be used as an alternative biomarker of clinical remission, providing another endpoint indicating a long-term response to NRTIs.

Various studies have shown/reported various degrees of HBsAg loss and/or different seroconversion rates but found differences between coinfections and mono-infections, with higher rates in cases of coinfection. Cumulative HBsAg seroclearance rates of 5% to 22% have been reported (Jaroszewicz et al., 2012; Kosi et al., 2012; Maylin et al., 2012; Zoutendijk et al., 2012; Hamers et al., 2013; Matthews et al., 2013; Boyd et al., 2015; Huang and Nunez, 2015; Boyd et al., 2016; Lucifora and Protzer, 2016; Price et al., 2017). A recent meta-analysis focused exclusively on the suppression of HBV with TDF-containing ART (Price et al., 2013). However, there have been few descriptions of HBeAg loss or seroconversion to anti-HBe, HBsAg loss, and the adverse effects of long-term treatment. Therefore, we performed this meta-analysis on data from patients with HIV/HBV coinfection to confirm the utility of HBsAg and HBeAg loss rates as biomarkers and to assess the seroconversion rates and determinants of HBsAg seroclearance during TDF-based treatment for the long-term follow-up of patients coinfected with HIV and HBV. We also considered the factors affecting HBsAg loss.

METHODS

This systematic review and meta-analysis were performed in accordance with PRISMA guidelines (Moher et al., 2009a; Moher et al., 2009b; Moher et al., 2009c), and the study is registered in the International Prospective Register of Systematic Reviews (PROSPERO, https://www.crd.york.ac.uk/PROSPERO/): CRD42018092379. The PRISMA checklist is included in **Supplementary Table S1**.

Search Strategy

A comprehensive literature search was performed in PubMed and Web of Science. The search terms used were intersections of treatment-related terms (TDF OR tenofovir) and disease terms (HIV OR AIDS OR HBV). Additional searches were also conducted in Google Scholar and ClinicalTrials.gov.

Selection Criteria

The inclusion criteria were as follows: 1) The study design had to be a randomized controlled trial (RCT) or prospective cohort study; 2) the treatment regimen had to contain TDF with or

without lamivudine (3TC) and/or emtricitabine (FTC); and 3) there had to be more than 10 participants in the TDF arm to prevent participant bias. We excluded 1) case reports; 2) review articles or theoretical articles; and 3) PhD theses, dissertations, and book chapters. Thus, to be more specific, 1) the participants were HIV-HBV coinfected patients at the screening stage of each study; 2) the eligible intervention contained TDF with or without 3TC and/or FTC, which are commonly used treatment combinations in most countries and regions; 3) some studies included in our meta-analysis were single-arm observational cohorts, while some studies compared the effectiveness between different treatment regimens; if the study arms used the medication combinations of interest, we included all arms; 4) because we aimed to investigate the immunological effects of targeted treatments, the outcomes of interest were HBV-related physiological processes during treatment. HBeAg and HBsAg are two key biomarkers for these processes; thus, we shifted our attention to the micro-level to detect the potential treatment efficacy. Two researchers independently performed the initial search, selecting studies on the basis of their titles and abstracts. The studies retained were then independently screened by a full-text assessment performed by the same researchers (TJ and TS). Disagreements between reviewers about study eligibility were resolved by discussion with BS. The procedure used for this study selection and the numbers of studies included and excluded are shown in **Figure 1**.

Data Extraction and Code

The data of interest were independently extracted by two researchers (TJ and WX). The outcomes of interest were HBsAg and HBeAg seroconversion. The additional information extracted from articles included article author(s), year of publication, study location, sample size, study design, treatment regimen, and disease-related variables.

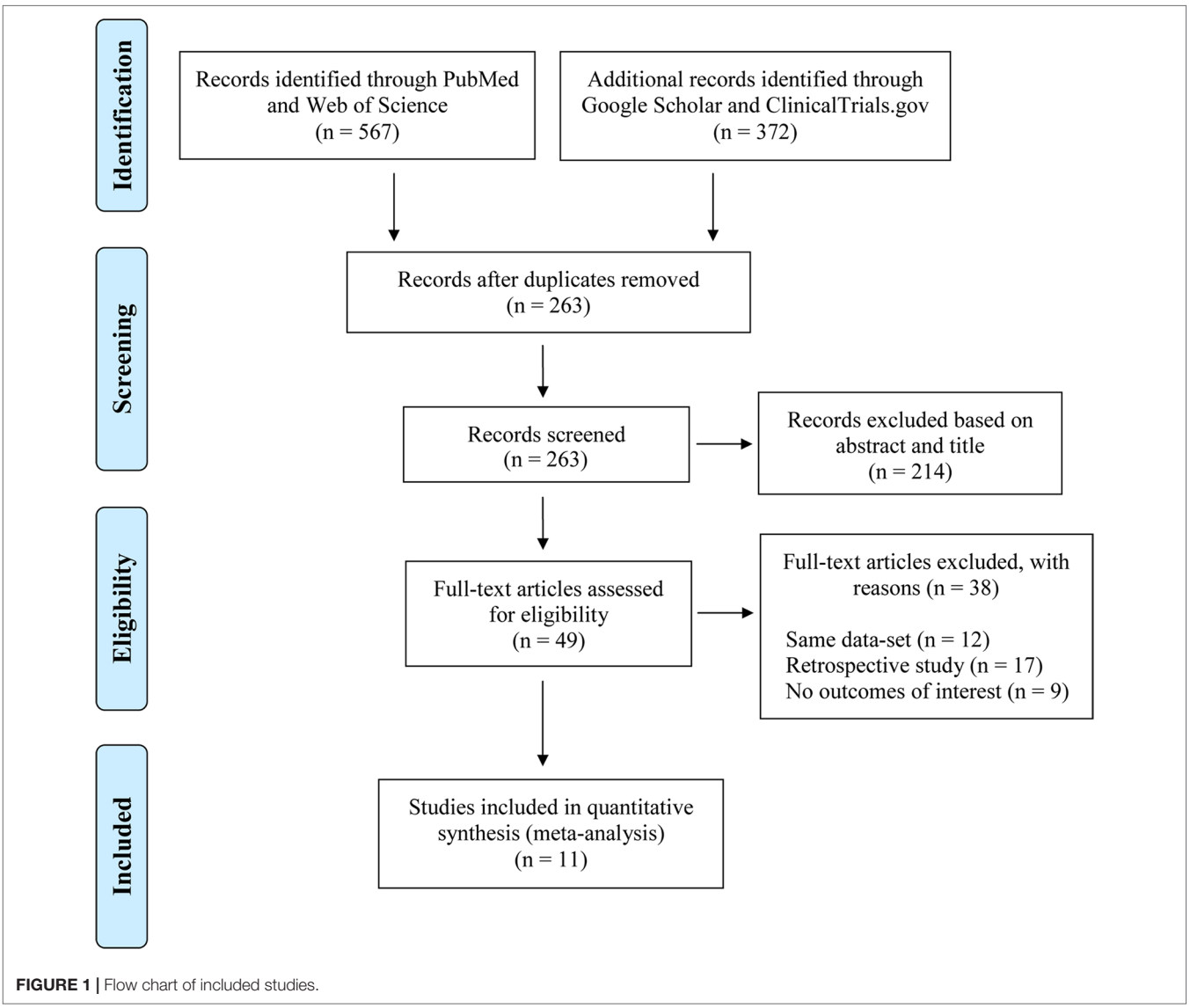

FIGURE 1 | Flow chart of included studies.

DATA ANALYSIS

We performed a quantitative analysis with Comprehensive Meta-Analysis (CMA) Version 2.0 (Biostat, Englewood, NJ, U.S.). We first calculated the combined event rate (ER) from the number of events and the sample size of the TDF-based treatment arm. A random effects meta-analysis was conducted with the ER to estimate HBeAg loss, HBeAg conversion, HBsAg loss, and HBsAg conversion. Thus, the ER in different figures denotes the pooled ERs for HBeAg loss, HBeAg conversion, HBsAg loss, and HBsAg conversion. The variation in effect size across studies was assessed by calculating the homogeneity statistic Q. The I^2 statistic was also used to estimate the proportion of heterogeneity in the observed variance (Higgins and Thompson, 2002). A subgroup meta-analysis was performed to assess the moderate effects of demographic and disease-related variables on HBsAg loss.

Study Quality and Publication Bias

The Newcastle-Ottawa Scale (NOS) was adopted to evaluate the study quality of the nonrandomized studies, and the Physiotherapy Evidence Database (PEDro) scale was used to assess the study quality of RCTs (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) (Moseley et al., 2002). The individual study quality for the included studies is shown in **Supplementary Table S2**.

Egger's intercept test and fail-safe N were used to assess publication bias across studies (Rosenthal, 1979; Stuck et al., 1998). The trim-and-fill method was used if significant publication bias was detected by Egger's test (Rosenthal, 1979). A subgroup meta-analysis was performed to assess the moderate effects of demographic and disease-related variables on HBsAg loss.

RESULTS

Characteristics of the Studies Included

We identified 11 studies eligible for this review, with sample sizes ranging from 10 to 100 (Dore et al., 2004; Stephan et al., 2005; Matthews et al., 2008; Nuesch et al., 2008; Avihingsanon et al., 2010; Hamers et al., 2013; Matthews et al., 2013; Huang and Nunez, 2015; Boyd et al., 2016; Li et al., 2016; Wu et al., 2016). We included three RCTs and eight prospective cohort studies. All participants were adults over the age of 18 years. The most commonly/frequently used ART regimen was TDF with 3TC or FTC. Five studies examined TDF-naive patients, whereas the other six studies examined treatment in 3TC-experienced patients. These studies reported various outcomes, including seroconversion for HBsAg and HBeAg. Detailed information about the studies included is provided in **Table 1**.

HBeAg Loss

The effect of TDF-containing treatment on HBeAg loss was reported for nine arms in eight studies. Therefore, it was possible to analyze the ER of each study. Egger's intercept test showed no significant publication bias (Kendall's tau = −1.556, $p = 0.264$), and the classic fail-safe N test showed that 63 missing studies would be required to obtain a non-significant result ($p > 0.05$). The combined ER for HBeAg loss was 0.249 (95% CI: 0.155–0.376, $p < 0.001$, **Figure 2**). There was significant heterogeneity across studies [$Q(8) = 18.092$, $p = 0.021$, $I^2 = 55.782$].

HBeAg Conversion

The effect of TDF-containing treatment on HBeAg conversion was reported for nine arms in eight studies, making it possible

TABLE 1 | Characteristics of the included studies.

Names	Location	Year of Publication	N	Study Design	Treatment Regimen	Baseline HBV RNA (log10 c/ml)	Baseline HIV RNA (log10 c/ml)	CD4 (cells/μl)	Duration (weeks)
Matthews	Thailand	2008	23	RCT	TDF+3TC[@]	8.4	4.7	39	48
					TDF[@]	8.6	5	25	48
Li	China	2016	91	Prospective	TDF+3TC [#]	3.49	4.7	229	48
Stephan	German	2005	31	Prospective	TDF-based[#]				48
Matthews	Thailand	2013	47	Prospective	3TC+TDF or TDF/FTC[@]	8.56	4.71	48	108
Dore	Western Europe, North America, Australia	2004	10	Prospective	TDF-based[#]	8.6	3.4	497	48
Wu	China	2016	100	Prospective	TDF+3TC [@#]	6.9	4.2	186.5	48
Huang	Taiwan	2016	89	Prospective	TDF-based[#]	6	4.7	361	144
Nuesch	Thailand	2008	16	RCT	TDF/FTC[@]	4.6	2.7	363	69
Avihingsanon	Netherlands Australia Thailand	2010	10	RCT	TDF/FTC[@]	8.54	4.9	69	48
Boyd	Côte d'Ivoire South Africa	2016	85	Prospective	TDF/FTC[@]				142
Hamers	South Africa, Zambia	2013	93	Prospective	TDF-based[@#]	5.18	4.91		48

[@]*TDF-naïve;* [#]*3TC-experience.*

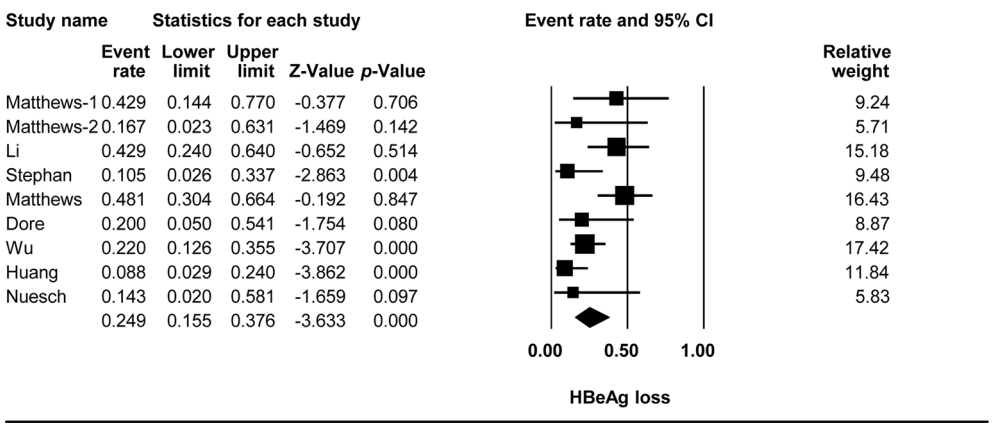

FIGURE 2 | Forest plot for HBeAg loss rates during TDF-containing treatment.

to analyze the ER of each of these studies. Egger's intercept test showed that there was no significant publication bias (Kendall's tau = −1.461, $p = 0.127$), and the classic fail-safe N tests showed that 70 missing studies would be required to obtain a non-significant result ($p > 0.05$). The combined ER for HBeAg loss was 0.237 (95% CI: 0.145–0.362, $p < 0.001$, **Figure 3**). There was significant heterogeneity across studies [Q(8) = 17.405, $p = 0.026$, $I^2 = 54.036$].

HBsAg Loss

The effect of TDF-containing treatment on HBsAg loss was reported for 10 arms in nine studies, so it was possible to analyze the ER of each study. Egger's intercept test showed that there was no significant publication bias (Kendall's tau = -0.667, $p = 0.617$), and the classic fail-safe N test showed that 356 missing studies would be required to obtain a non-significant result ($p > 0.05$). The combined ER for HBsAg loss was 0.073 (95% CI: 0.044–0.119, $p < 0.001$, **Figure 4**). There was no significant heterogeneity across studies [Q(9) = 14.433, $p = 0.108$, $I^2 = 37.641$].

Three studies with four arms were performed in Asia, and the other six studies were performed elsewhere. Stratification based on the location showed that location had a significant effect on the TDF-containing regimens [Q(1) = 5.233, $p = 0.022$, Asia vs. other countries: 0.037 (95% CI: 0.018–0.077) vs. 0.099 (95% CI: 0.058–0.164)].

Two studies included patients with $CD4^+$ T-cell counts of at least 200 cells/μl, whereas five studies included patients with fewer than 200 cells/μl. Stratification based on the baseline $CD4^+$ T-cell counts revealed an effect of marginal significance on the efficacy of TDF-containing regimens [Q(1) = 3.095, $p = 0.078$, 200 or more cells/μl vs. fewer than 200 cells/μl: 0.039 95% CI: 0.015–0.098 vs. 0.094 95% CI (0.026–0.218)].

In five studies with six arms, 3TC was used before TDF, whereas in three studies, TDF was used in patients not previously exposed to 3TC. Prior exposure to 3TC significantly affected the efficacy of TDF-containing regimens [Q(1) = 4.204, $p = 0.04$, yes vs. no: 0.041 95% CI (0.017-0.099) vs. 0.109 95% CI (0.068–0.169)].

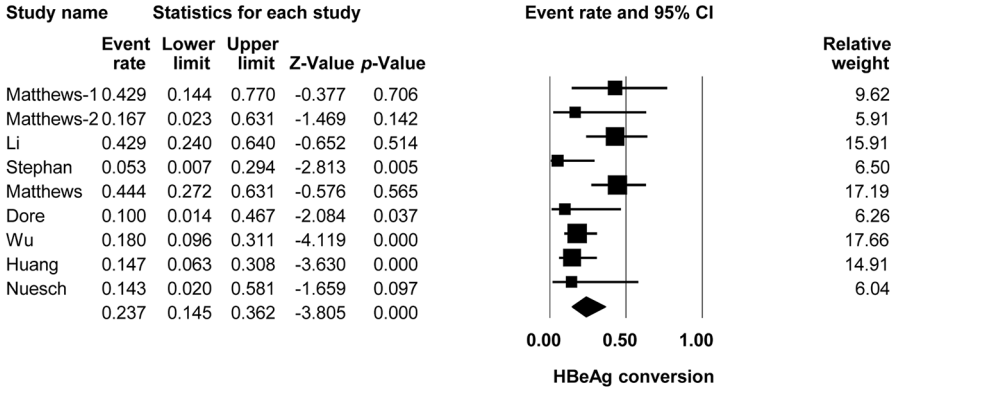

FIGURE 3 | Forest plot for HBeAg conversion rates during TDF-containing treatment.

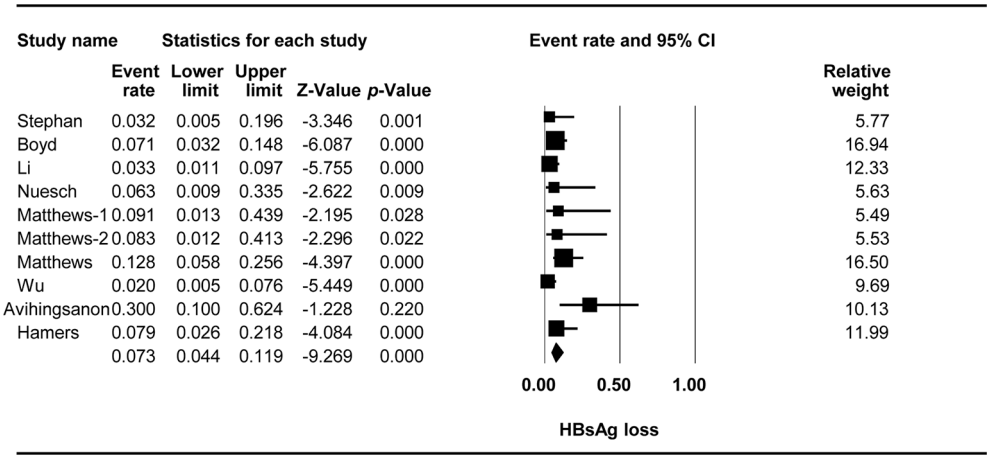

FIGURE 4 | Forest plot for HBsAg loss rates during TDF-containing treatment.

Six studies reported detectable HBV at the baseline, whereas three studies with four arms reported undetectable HBV at the baseline. The baseline HBV viral load significantly affected the efficacy of TDF-containing regimens [Q(1) = 7.938, p = 0.005, yes vs. no: 0.147 95% CI (0.083–0.247) vs. 0.05 95% CI (0.031–0.08)].

HBsAg Conversion

The effect of TDF-containing treatments on HBsAg loss was reported in nine studies with 10 arms, making it possible to analyze the ER of each of these studies. Egger's intercept test showed that there was no significant publication bias (Kendall's tau = −0.753, p = 0.886), and the classic fail-safe N test showed that 56 missing studies would be required to obtain a non-significant result ($p > 0.05$). The combined ER for HBeAg loss was 0.055 (95% CI: 0.02–0.142, $p < 0.001$, **Figure 5**). There was no significant heterogeneity across studies [Q(3) = 5.81, p = 0.121, I^2 = 48.365].

DISCUSSION

This systematic review and meta-analysis provides pooled estimates of the serological outcomes of TDF-containing regimens in patients with HIV-HBV coinfection. The overall estimates are useful for targeted treatments in key populations.

We found that almost a quarter of the participants experienced serological changes in HBeAg. However, these results should be interpreted with caution due to a relatively high degree of heterogeneity among the studies included. The rates of HBeAg loss ranged from 8.8% to 48%, and the rates of HBeAg seroconversion ranged from 10% to 44%. The causes of this heterogeneity should be identified, as long-term treatment resulted in higher rates of HBeAg loss and seroconversion. The combination of TDF + 3TC/FTC seemed to be more effective in patients with documented 3TC resistance (Luo et al., 2018). Differences in immune restoration after ART initiation were observed, with various rates of HBeAg seroclearance, as a sudden increase in $CD4^+$ T-cell counts may promote a rapid immune response (Miailhes et al., 2007).

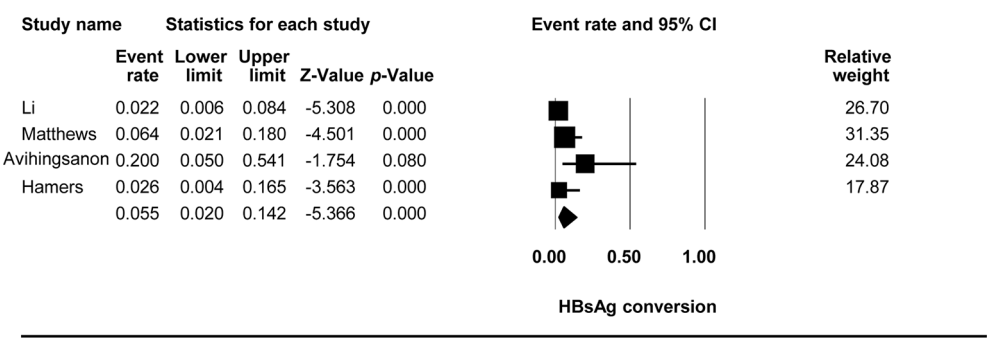

FIGURE 5 | Forest plot for HBsAg conversion rates during TDF-containing treatment.

HBsAg loss was observed in 7.3% of the coinfected patients, and the HBV curve was recorded in 5.5% of participants, which was consistent with the results of previous observational studies (Martin-Carbonero et al., 2011; Van Griensven et al., 2014). Understanding the predictors of HBsAg loss is an important research priority in the search for novel strategies for achieving HBV remission, and individuals with HIV-HBV coinfection may constitute a unique group for studying such associations.

Minor immunosuppression appeared to influence the baseline HBsAg and HBeAg levels, with a negative impact on the decrease in HBsAg and HBeAg levels. In our review, we found that patients with $CD4^+$ T-cell counts below 200 cells/μl and higher HBV DNA levels at the baseline were more likely to display HBsAg loss than were their counterparts. This might reflect robust immune reconstitution and the acquisition of enhanced pathogen-specific innate or adaptive immune responses following treatment with TDF-containing regimens (Hsu et al., 2012; Boyd et al., 2015). However, a previous study showed that patients with $CD4^+$ T-cell counts below 300 cells/μl during TDF-containing treatment, a level considered to constitute mild immunosuppression, cannot achieve a strong enough immune response to clear infected hepatocytes (De Vries-Sluijs et al., 2010).

Higher levels of HBV DNA and exposure to 3TC are associated with longer times before the achievement of undetectable levels of HBV DNA while receiving TDF (Childs et al., 2013), and a longer time to the occurrence of an immunological response represented by HBsAg loss. Therefore, studies in real-world settings are required to determine the effects on HBsAg and HBeAg seroclearance in patients with prior 3TC treatment and patients receiving TDF-based ART as the initial treatment.

We detected differences between Asia and other parts of the world, with HIV-induced immunosuppression associated with different degrees of HBsAg loss in patients with HBV-HIV coinfection. Many studies have shown that genotype A is the most prevalent HBV genotype in non-Asian populations, which display higher levels of HBeAg and HBsAg during natural infections and following IFN treatment (Erhardt et al., 2005; Flink et al., 2006; Thio and Locarnini, 2007). The HBV genotype distribution is different in Asia and other regions; thus, the subtypes of HBV and their virological responses require further investigation.

Although the safety of TDF-containing regimens was not our primary outcome of interest in this meta-analysis, substantial attention should be paid by health providers and policymakers to monitoring potential side effects in people living with HIV-HBV coinfection. An elevated incidence of renal and liver dysfunction was detected among HIV-HBV coinfected participants on long-term TDF treatment in recent studies (Peters et al., 2006; Tan et al., 2009). Although few studies have reported potential side effects on bone mineral density among HIV-HBV coinfected patents, a recent systematic review and meta-analysis among people mono-infected with HBV or HIV have reported reduced bone density and an elevated incidence of bone fracture due to long-term use of TDF-containing treatments (Buti et al., 2018; Goh et al., 2018). Thus, considering the high availability and low cost of TDF-based treatment in middle- or low-income settings among HIV-HBV coinfected participants, it is better to closely monitor and promptly treat these side effects. In addition, in developed or high-income countries, clinicians may choose treatment regimens with fewer side effects, such as tenofovir alafenamide (TAF)-based treatment regimens.

This review has several limitations. First, the results should be interpreted with caution due to the limited number of comparisons included, potentially restricting the external validity of the conclusions in other settings and decreasing the statistical power to detect potentially significant results. Second, the relatively high level of heterogeneity may have decreased the representativeness of some outcomes (i.e., HBeAg loss and conversion). Third, only a few studies reported adverse events related to kidney and liver function, and this may limit attempts to quantitatively assess the safety of TDF-containing regimens in patients with HIV-HBV coinfection.

CONCLUSION

This systematic review and meta-analysis demonstrated that TDF-containing regimens are effective at stimulating HBeAg loss (24.9%), HBeAg conversion (23.7%), HBsAg loss (7.3%), and HBsAg conversion (5.5%) in HIV-HBV coinfected patients. The moderator analysis showed that Asian ethnicity, prior exposure to 3TC, and nondetectable baseline HBV viral load are associated with lower odds of HBsAg loss. However, we should cautiously interpret the results regarding HBeAg loss and HBeAg conversion due to significant heterogeneity across all study arms. Well-designed prospective cohort studies and RCTs with large sample sizes are required for the investigation of potential predictors and biological markers associated with strategies for achieving HBV remission in patients with HIV-HBV coinfection, which is a matter of considerable importance to clinicians and those responsible for health policies.

AUTHOR CONTRIBUTIONS

TJ, BS, and HW conceived and designed the protocol and study. TJ, ZZ, WX, TZ, and HW identified studies to be screened. TJ, BS, TS, LD, WW, and TZ identified studies for eligibility, extracted data, and assessed the methodological quality of included studies. TJ and BS wrote the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Aidsinfo. Panel on antiretroviral guidelines for adults and adolescents. *Guidelines for the use of antiretroviral agents in adults and adolescents living with HIV*. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf.

Avihingsanon, A., Lewin, S. R., Kerr, S., Chang, J. J., Piyawat, K., Napissanant, N., et al. (2010). Efficacy of tenofovir disoproxil fumarate/emtricitabine compared with emtricitabine alone in antiretroviral-naive HIV-HBV coinfection in Thailand. *Antiviral Therapy* 15, 917–922. doi: 10.3851/IMP1645

Bihl, F., Martinetti, G., Wandeler, G., Weber, R., Ledergeber, B., Calmy, A., et al. (2015). HBV genotypes and response to tenofovir disoproxil fumarate in HIV/HBV-coinfected persons. *BMC Gastroenterol.* 15, 79. doi: 10.1186/s12876-015-0308-0

Boyd, A., Gozlan, J., Miailhes, P., Lascoux-Combe, C., Cam, M. S., Rougier, H., et al. (2015). Rates and determinants of hepatitis B 'e' antigen and hepatitis B surface antigen seroclearance during long-term follow-up of patients coinfected with HIV and hepatitis B virus. *AIDS* 29, 1963–1973. doi: 10.1097/QAD.0000000000000795

Boyd, A., Maylin, S., Moh, R., Mahjoub, N., Gabillard, D., Eholie, S. P., et al. (2016). Hepatitis B surface antigen quantification as a predictor of seroclearance during treatment in HIV-hepatitis B virus coinfected patients from Sub-Saharan Africa. *J. Gastroenterol. Hepatol.* 31, 634–644. doi: 10.1111/jgh.13156

Buti, M., Riveiro-Barciela, M., and Esteban, R. (2018). Long-term safety and efficacy of nucleo(t)side analogue therapy in hepatitis B. *Liver Int.* 38 Suppl 1, 84–89. doi: 10.1111/liv.13641

Childs, K., Joshi, D., Byrne, R., Bruce, M., Carey, I., Agarwal, K., et al. (2013). Tenofovir-based combination therapy for HIV/HBV co-infection: factors associated with a partial HBV virological response in patients with undetectable HIV viraemia. *AIDS* 27, 1443–1448. doi: 10.1097/QAD.0b013e32836011c2

Coffin, C. S., Osiowy, C., Myers, R. P., and Gill, M. J. (2013). Virology and clinical sequelae of long-term antiviral therapy in a North American cohort of hepatitis B virus (HBV)/human immunodeficiency virus type 1 (HIV-1) co-infected patients. *J. Clin. Virol.* 57, 103–108. doi: 10.1016/j.jcv.2013.02.004

De Vries-Sluijs, T. E., Reijnders, J. G., Hansen, B. E., Zaaijer, H. L., Prins, J. M., Pas, S. D., et al. (2010). Long-term therapy with tenofovir is effective for patients co-infected with human immunodeficiency virus and hepatitis B virus. *Gastroenterology* 139, 1934–1941. doi: 10.1053/j.gastro.2010.08.045

Dore, G. J., Cooper, D. A., Pozniak, A. L., Dejesus, E., Zhong, L., Miller, M. D., et al. (2004). Efficacy of tenofovir disoproxil fumarate in antiretroviral therapy-naive and -experienced patients coinfected with HIV-1 and hepatitis B virus. *J. Infect. Dis.* 189, 1185–1192. doi: 10.1086/380398

Erhardt, A., Blondin, D., Hauck, K., Sagir, A., Kohnle, T., Heintges, T., et al. (2005). Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut* 54, 1009–1013. doi: 10.1136/gut.2004.060327

Flink, H. J., Van Zonneveld, M., Hansen, B. E., De Man, R. A., Schalm, S. W., and Janssen, H. L. (2006). Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. *Am J. Gastroenterol.* 101, 297–303. doi: 10.1111/j.1572-0241.2006.00418.x

Goh, S. S. L., Lai, P. S. M., Tan, A. T. B., and Ponnampalavanar, S. (2018). Reduced bone mineral density in human immunodeficiency virus-infected individuals: a meta-analysis of its prevalence and risk factors: supplementary presentation. *Osteoporos. Int.* 29, 1683. doi: 10.1007/s00198-018-4379-y

Hamers, R. L., Zaaijer, H. L., Wallis, C. L., Siwale, M., Ive, P., Botes, M. E., et al. (2013). HIV-HBV coinfection in Southern Africa and the effect of lamivudine-versus tenofovir-containing cART on HBV outcomes. *J. Acquir. Immune Defic. Syndr.* 64, 174–182. doi: 10.1097/QAI.0b013e3182a60f7d

Higgins, J. P., and Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21, 1539–1558. doi: 10.1002/sim.1186

Hsu, D. C., Zaunders, J. J., Plit, M., Leeman, C., Ip, S., Iampornsin, T., et al. (2012). A novel assay detecting recall response to Mycobacterium tuberculosis: comparison with existing assays. *Tuberculosis (Edinb)* 92, 321–327. doi: 10.1016/j.tube.2012.03.008

Http://Www.Ohri.Ca/Programs/Clinical_Epidemiology/Oxford.Asp.

Huang, A. J., and Nunez, M. (2015). Outcomes in HIV/HBV-coinfected patients in the tenofovir era are greatly affected by immune suppression. *J. Int. Assoc. Provid. AIDS Care* 14, 360–368. doi: 10.1177/2325957415586258

Jaroszewicz, J., Reiberger, T., Meyer-Olson, D., Mauss, S., Vogel, M., Ingiliz, P., et al. (2012). Hepatitis B surface antigen concentrations in patients with HIV/HBV co-infection. *PLoS One* 7, e43143. doi: 10.1371/journal.pone.0043143

Kitrinos, K. M., Corsa, A., Liu, Y., Flaherty, J., Snow-Lampart, A., Marcellin, P., et al. (2014). No detectable resistance to tenofovir disoproxil fumarate after 6 years of therapy in patients with chronic hepatitis B. *Hepatology* 59, 434–442. doi: 10.1002/hep.26686

Kosi, L., Reiberger, T., Payer, B. A., Grabmeier-Pfistershammer, K., Strassl, R., Rieger, A., et al. (2012). Five-year on-treatment efficacy of lamivudine-, tenofovir- and tenofovir + emtricitabine-based HAART in HBV-HIV-coinfected patients. *J. Viral. Hepat.* 19, 801–810. doi: 10.1111/j.1365-2893.2012.01601.x

Li, Y., Xie, J., Han, Y., Wang, H., Zhu, T., Wang, N., et al. (2016). Lamivudine monotherapy-based cART is efficacious for HBV treatment in HIV/HBV coinfection when baseline HBV DNA <20,000 IU/mL. *J. Acquir. Immune Defic. Syndr* 72, 39–45. doi: 10.1097/QAI.0000000000000927

Lucifora, J., and Protzer, U. (2016). Attacking hepatitis B virus cccDNA—the holy grail to hepatitis B cure. *J. Hepatol.* 64, S41–S48. doi: 10.1016/j.jhep.2016.02.009

Luo, A., Jiang, X., and Ren, H. (2018). Lamivudine plus tenofovir combination therapy versus lamivudine monotherapy for HBV/HIV coinfection: a meta-analysis. *Virol. J.* 15, 139. doi: 10.1186/s12985-018-1050-3

Martin-Carbonero, L., Teixeira, T., Poveda, E., Plaza, Z., Vispo, E., Gonzalez-Lahoz, J., et al. (2011). Clinical and virological outcomes in HIV-infected patients with chronic hepatitis B on long-term nucleos(t)ide analogues. *AIDS* 25, 73–79. doi: 10.1097/QAD.0b013e328340fde2

Matthews, G. V., Ali, R. J., Avihingsanon, A., Amin, J., Hammond, R., Bowden, S., et al. (2013). Quantitative HBsAg and HBeAg predict hepatitis B seroconversion after initiation of HAART in HIV-HBV coinfected individuals. *PLoS One* 8, e61297. doi: 10.1371/journal.pone.0061297

Matthews, G. V., Avihingsanon, A., Lewin, S. R., Amin, J., Rerknimitr, R., Petcharapirat, P., et al. (2008). A randomized trial of combination hepatitis B therapy in HIV/HBV coinfected antiretroviral naive individuals in Thailand. *Hepatology* 48, 1062–1069. doi: 10.1002/hep.22462

Maylin, S., Boyd, A., Lavocat, F., Gozlan, J., Lascoux-Combe, C., Miailhes, P., et al. (2012). Kinetics of hepatitis B surface and envelope antigen and prediction of treatment response to tenofovir in antiretroviral-experienced HIV-hepatitis B virus-infected patients. *AIDS* 26, 939–949. doi: 10.1097/QAD.0b013e328352224d

Miailhes, P., Trabaud, M. A., Pradat, P., Lebouche, B., Chevallier, M., Chevallier, P., et al. (2007). Impact of highly active antiretroviral therapy (HAART) on the

natural history of hepatitis B virus (HBV) and HIV coinfection: relationship between prolonged efficacy of HAART and HBV surface and early antigen seroconversion. *Clin. Infect. Dis.* 45, 624–632. doi: 10.1086/520752

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009a). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann. Intern. Med.* 151, 264–269, W264. doi: 10.7326/0003-4819-151-4-200908180-00135

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009b). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 6, e1000097. doi: 10.1371/journal.pmed.1000097

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009c). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 339, b2535. doi: 10.1136/bmj.b2535

Moseley, A. M., Herbert, R. D., Sherrington, C., and Maher, C. G. (2002). Evidence for physiotherapy practice: a survey of the Physiotherapy Evidence Database (PEDro). *Aust. J. Physiother.* 48, 43–49. doi: 10.1016/S0004-9514(14)60281-6

Nuesch, R., Ananworanich, J., Srasuebkul, P., Chetchotisakd, P., Prasithsirikul, W., Klinbuayam, W., et al. (2008). Interruptions of tenofovir/emtricitabine-based antiretroviral therapy in patients with HIV/hepatitis B virus co-infection. *AIDS* 22, 152–154. doi: 10.1097/QAD.0b013e3282f303bf

Nunez, M., Perez-Olmeda, M., Diaz, B., Rios, P., Gonzalez-Lahoz, J., and Soriano, V. (2002). Activity of tenofovir on hepatitis B virus replication in HIV-co-infected patients failing or partially responding to lamivudine. *AIDS* 16, 2352–2354. doi: 10.1097/00002030-200211220-00023

Peters, M. G., Andersen, J., Lynch, P., Liu, T., Alston-Smith, B., Brosgart, C. L., et al. (2006). Randomized controlled study of tenofovir and adefovir in chronic hepatitis B virus and HIV infection: ACTG A5127. *Hepatology* 44, 1110–1116. doi: 10.1002/hep.21388

Price, H., Dunn, D., Pillay, D., Bani-Sadr, F., De Vries-Sluijs, T., Jain, M. K., et al. (2013). Suppression of HBV by tenofovir in HBV/HIV coinfected patients: a systematic review and meta-analysis. *PLoS One* 8, e68152. doi: 10.1371/journal.pone.0068152

Price, H., Dunn, D., Zachary, T., Vudriko, T., Chirara, M., Kityo, C., et al. (2017). Hepatitis B serological markers and plasma DNA concentrations. *AIDS* 31, 1109–1117. doi: 10.1097/QAD.0000000000001454

Ristig, M. B., Crippin, J., Aberg, J. A., Powderly, W. G., Lisker-Melman, M., Kessels, L., et al. (2002). Tenofovir disoproxil fumarate therapy for chronic hepatitis B in human immunodeficiency virus/hepatitis B virus-coinfected individuals for whom interferon-alpha and lamivudine therapy have failed. *J. Infect. Dis.* 186, 1844–1847. doi: 10.1086/345770

Rosenthal, R. (1979). The file drawer problem and tolerance for null results. *Psychological Bulletin* 86, 638–641. doi: 10.1037/0033-2909.86.3.638

Stephan, C., Berger, A., Carlebach, A., Lutz, T., Bickel, M., Klauke, S., et al. (2005). Impact of tenofovir-containing antiretroviral therapy on chronic hepatitis B in a cohort co-infected with human immunodeficiency virus. *J. Antimicrob. Chemother.* 56, 1087–1093. doi: 10.1093/jac/dki396

Stuck, A. E., Rubenstein, L. Z., and Wieland, D. (1998). Bias in meta-analysis detected by a simple, graphical test. Asymmetry detected in funnel plot was probably due to true heterogeneity. *BMJ* 316, 469. author reply 470–461. doi: 10.1136/bmj.316.7129.469

Tan, L. K., Gilleece, Y., Mandalia, S., Murungi, A., Grover, D., Fisher, M., et al. (2009). Reduced glomerular filtration rate but sustained virologic response in HIV/hepatitis B co-infected individuals on long-term tenofovir. *J. Viral. Hepat.* 16, 471–478. doi: 10.1111/j.1365-2893.2009.01084.x

Thio, C. L., and Locarnini, S. (2007). Treatment of HIV/HBV coinfection: clinical and virologic issues. *AIDS Rev* 9, 40–53. doi: 10.1089/aid.2007.9999

Tseng, T. C., Liu, C. J., Yang, H. C., Su, T. H., Wang, C. C., Chen, C. L., et al. (2012). High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. *Gastroenterology* 142, 1140–1149 e1143. doi: 10.1053/j.gastro.2012.02.007

Unaids (2018). Global HIV & AIDS statistics—2018 fact sheet. WORLD AIDS DAY 2018.

Van Griensven, J., Phirum, L., Choun, K., Thai, S., De Weggheleire, A., and Lynen, L. (2014). Hepatitis B and C co-infection among HIV-infected adults while on antiretroviral treatment: long-term survival, CD4 cell count recovery and antiretroviral toxicity in Cambodia. *PLoS One* 9, e88552. doi: 10.1371/journal.pone.0088552

Vinikoor, M. J., Sinkala, E., Chilengi, R., Mulenga, L. B., Chi, B. H., Zyambo, Z., et al. (2017). Impact of antiretroviral therapy on liver fibrosis among human immunodeficiency virus-infected adults with and without HBV coinfection in Zambia. *Clin. Infect. Dis.* 64, 1343–1349. doi: 10.1093/cid/cix122

Wu, Y. S., Zhang, W. W., Ling, X. M., Yang, L., Huang, S. B., Wang, X. C., et al. (2016). Efficacy and safety of tenofovir and lamivudine in combination with efavirenz in patients co-infected with human immunodeficiency virus and hepatitis B virus in China. *Chin. Med. J. (Engl)* 129, 304–308. doi: 10.4103/0366-6999.174509

Yang, Y., Gao, J., Li, H. L., Zheng, W., Yang, G., Zhang, W., et al. (2016). Dose-response association between hepatitis B surface antigen levels and liver cancer risk in Chinese men and women. *Int J Cancer* 139, 355–362. doi: 10.1002/ijc.30086

Zeisel, M. B., Lucifora, J., Mason, W. S., Sureau, C., Beck, J., Levrero, M., et al. (2015). Towards an HBV cure: state-of-the-art and unresolved questions—report of the ANRS workshop on HBV cure. *Gut* 64, 1314–1326. doi: 10.1136/gutjnl-2014-308943

Zoutendijk, R., Zaaijer, H. L., De Vries-Sluijs, T. E., Reijnders, J. G., Mulder, J. W., Kroon, F. P., et al. (2012). Hepatitis B surface antigen declines and clearance during long-term tenofovir therapy in patients coinfected with HBV and HIV. *J. Infect. Dis.* 206, 974–980. doi: 10.1093/infdis/jis439

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Transcriptional Analysis of the Effects of Gambogic Acid and Neogambogic Acid on Methicillin-Resistant *Staphylococcus aureus*

*Xin Hua[1,2], Yue Jia[1], Qin Yang[1], Wanjiang Zhang[1], Zhimin Dong[3] and Siguo Liu[1]**

[1] Division of Bacterial Diseases, State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, China, [2] College of Life Science, Northeast Forestry University, Harbin, China, [3] Innovation Team of Livestock and Poultry Epidemic Disease Prevention and Control, Tianjin Animal Science and Veterinary Research Institute, Tianjin, China

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is a major threat to human health, as this bacterium has developed resistance to a variety of conventional antibiotics. This is especially true of MRSA biofilms, which not only exhibit enhanced pathogenicity but also are resistant to most antibiotics. In this work, we demonstrated that two natural products with antitumor activity, namely, gambogic acid (GA) and neogambogic acid (NGA), have significant inhibitory activity toward MRSA. GA and NGA can not only effectively inhibit planktonic MRSA strains *in vivo* and *in vitro,* but also have strong inhibitory effects on MRSA biofilms formation. By transcriptome sequencing, Q-RT-PCR and PRM, we found that GA and NGA could reduce the expression of *S. aureus* virulence factors by inhibiting the *saeRS* two-component, thus achieving inhibition of MRSA. We found that GA and NGA had anti-MRSA activity *in vivo* and *in vitro* and identified *saeRS* to be the target, indicating that *saeRS* inhibitors may be used to treat biofilm-related infections.

Keywords: MRSA, *staphylococcus aureus* biofilms, saeRS two-component system, gambogic acid, neogambogic acid

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Kwame Nkrumah University of Science and Technology, Ghana

***Correspondence:**
Siguo Liu
liusiguo@caas.cn

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) can induce multiple human diseases, such as necrotic pneumonia, endocarditis, and septicemia (Tenover and Goering, 2009; Alam et al., 2015; David and Daum, 2017). In the United States, it is estimated that the mortality rate due to MRSA infection is higher than that of HIV/AIDS and tuberculosis (Infectious Diseases Society of America et al., 2011); nearly 11,000 people die each year from MRSA infection (Mohammad et al., 2015; Thangamani et al., 2015b). MRSA is considered to be a major public health concern in hospital and community settings (Tavares et al., 2014; Lehar et al., 2015; Udo and Al-Sweih, 2017). Due to significant resistance of MRSA to a wide range of antibiotics, treatment tends to be ineffective, especially after biofilm formation, which limits the number of therapeutic options available (Pozzi et al., 2012; Ohadian Moghadam et al., 2014; Vazquez-Sanchez et al., 2018). MRSA is a challenge

Abbreviations: MRSA, *Staphylococcus aureus*; GA, Gambogic acid; NGA, Neogambogic acid; TCS, Two-component signaling; MIC, Minimal inhibitory concentration; MBC, Minimum bactericidal concentration; PVL, Panton-Valentine leukocidin; PRM, Parallel reaction monitoring; SEM, Scanning electron microscopy; GlcNAc, N-acetylglucosamine; MurNAc, N-acetylmuramic acid.

for the medical field worldwide, and antibiotics remains the major method of treatment. Regrettably, it takes a long time to develop new antibiotics, and antibiotic development has not been able to keep pace with the emergence of new generations of resistant bacteria. Hence, the development of novel therapeutic agents and antibiotic substitutes with activity against highly pathogenic bacteria is urgently required.

The high pathogenicity and mortality rate due to *S. aureus* infection are mainly attributed to the various virulence factors produced by this bacterium (Ferro et al., 2016). These secreted toxins are associated with host tissue infection, immune evasion and bacterial pathogenesis (Miyazaki et al., 2012; Den Reijer et al., 2016; Ferro et al., 2016). MRSA toxins and biofilms directly affect wound healing in patients, leading to further systemic complications (Smith et al., 2010; Federman et al., 2016). In *S. aureus*, the expression of these virulence factors is controlled by a network of transcription factors (such as *mgrA*, *sarA*, *sigB,* and *rot*) and two-component regulatory systems (such as *srrAB*, *arlRS*, *vraSR,* and *saeRS*) (Boyle-Vavra et al., 2006; Cho et al., 2015; Guo et al., 2017). As a major signal transduction mechanism in bacteria, two-component signaling (TCS) is responsible for adaptation to environmental changes *via* the sensing of various cues (such as nutrient concentration, ionic strength, and membrane interference) (Giraudo et al., 1999; Fournier and Hooper, 2000; Hall et al., 2017). The *saeRS* two-component system plays a vital role in the expression and pathogenesis of *Staphylococcus* virulence genes and can regulate more than 20 virulence factors, such as coagulase, alpha-hemolysin and fibronectin-binding proteins. Although the *saeRS* two-component system has been reported to be directly associated with the formation of *S. aureus* biofilms, drugs targeting *saeRS* have not been developed (Cho et al., 2015; Liu et al., 2016; Guo et al., 2017).

GA and NGA are two active compounds found in *Garcinia* species, which exhibit immune-enhancing, anti-inflammatory, antitumor, and proapoptotic activities (Wang et al., 2011; Chen et al., 2015; Zhang et al., 2016; Jin et al., 2018). Especially in the aspects of anti-inflammatory and anti-tumor, it has been found that gamoic acid can inhibit many cell signaling pathways, such as nuclear factor-kappa B (nf-κb), tumor necrosis factor-α (TNF-α), and iNOS (Pandey et al., 2016; Sun et al., 2018). It has been reported that a series of xanthone derivatives, including GA, have anti-MRSA strain activity, and could disrupt intracellular invasion of *S. aureus*, but no further research has been conducted on the mechanism of action (Chaiyakunvat et al., 2016). In addition, there are few reports on the antibacterial activities of GA or NGA.

In this paper, we demonstrated the inhibition of MRSA and the activity against biofilm formation by GA and NGA *in vivo* and *in vitro*. This antibacterial activity is mainly achieved by inhibiting the expression of multiple virulence factors in MRSA, which in turn occurs *via* inhibition of the *saeRS* two-component system. In this study, we reported for the first time that GA and NGA have the activity of inhibiting MRSA biofilm formation, and revealed the new mechanism of the antimicrobial activity of GA and NGA. This study provides favorable evidence for the study of the anti-bacterial mechanism of GA and NGA.

MATERIALS AND METHODS

Strains and Growth Conditions

Clinical MRSA and MSSA isolates were kindly donated by the First Affiliated Hospital of Harbin Medical University, Harbin, China. The *S. aureus* standard strains ATCC29213 (methicillin-sensitive *staphylococcus aureus*) and ATCC 33591 (MRSA) (American Type Culture Collection, USA) and the clinical MRSA strain maintained in Mueller-Hinton broth (MHB, Oxoid, Basingstoke, England) were frozen at −80°C before use. Details regarding the strains have been provided in previous reports (Hua et al., 2018).

Antimicrobial Agents

GA, NGA, vancomycin, and linezolid were purchased from Sigma Aldrich (Bornem, Belgium) , the structural formula of GA and NGA are shown in **Figure 1**. GA and NGA were dissolved in DMSO. Vancomycin and linezolid were dissolved in ultrapure water.

MIC and MBC Measurement

Based on the CLSI guidelines, broth micro dilution was adopted to determine the MIC and MBC values. Briefly, for MIC and MBC determination, the test medium was Trypticase soy broth (TSB) and the density of bacteria was 5×10^5 colony forming units (CFU)/mL. Cell suspensions (200 μL) were inoculated into the wells with antibiotics at different final concentrations (32, 16, 8, 4, 2, 1, 0.5, and 0.25 mg/mL). The inoculated microplates were incubated at 37°C for 16 h before being read. The MIC and MBC were interpreted as the lowest concentration of antibiotic that completely inhibited the visible growth or killed the bacteria. The experiment was carried out in triplicate.

Cytotoxicity Assay

HeLa cells and mouse skin keratinocytes cells (CP-M168, form Procell Life Science&Technology Co., Ltd. Wuhan, China) were seeded at a density of 10,000 cells per well in a 96-well cell culture plate (NEST, Nest Biotech Co., Ltd., NJ, USA) and incubated overnight at 37°C in dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS). Then, the cells were treated with GA and NGA for 24 h at different concentrations from 0 to 128 μg/mL. The treated cells were washed four times with PBS, and DMEM containing MTS (20%) assay reagent (3-(4,5-dimethylthiazol-2-yl)-5-(3carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; Promega, Madison, WI, USA) was added. After 4 h of incubation at 37°C, the absorbance was measured using an ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA). The percent cell viability of the GA- and NGA-treated cells was calculated.

$$\text{cell viability } 100\% = \frac{0\text{Dtreated group}}{0\text{Dcontrol group}}$$

Time-Dependent Killing

An overnight cell culture (*S. aureus* ATCC33591, about 1×10^{10}) was diluted 1:5,000 in MHB and incubated at 37°C

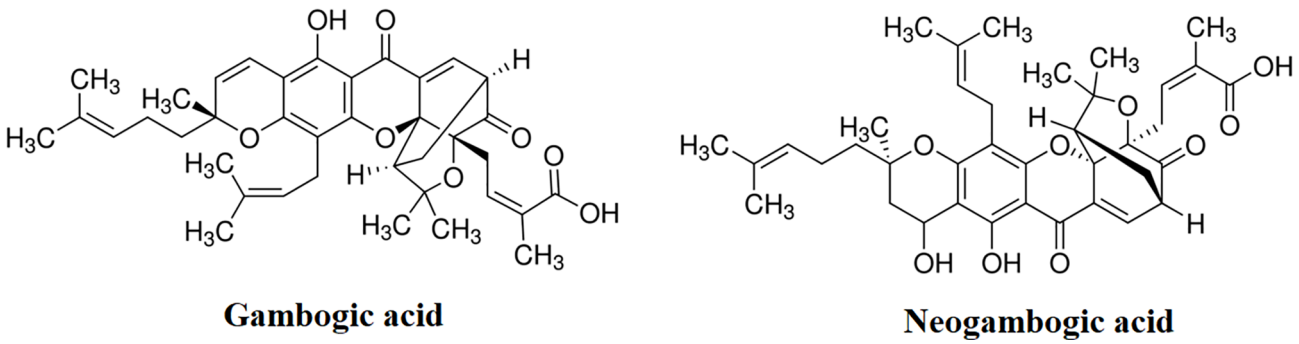

FIGURE 1 | Chemical structure of gambogic acid and neogambogic acid. (https://www.sigmaaldrich.com/catalog/product/sigma/g8171?lang=zh&region=CN; https://www.sigmaaldrich.com/catalog/product/supelco/phl83527?lang=zh&region=CN)

and 220 rpm for 2 h. Then, the bacterial cells were treated with GA, NGA or vancomycin at a concentration of 5 × MIC. One milliliter of each culture was removed at specific time intervals and centrifuged at 10,000 × g for 1 min. The pellet was resuspended in 100 mL of sterile PBS. Diluted suspensions were plated on Mueller-Hinton agar (MHA) and incubated at 37°C overnight for CFU calculation. Experiments were performed with three replicates.

Virulence Factor Detection

The effects of GA, NGA, linezolid, and vancomycin on the production of two important *S. aureus* toxins (Hla and Panton-Valentine leukocidin (PVL)) was measured by utilizing ELISA as described previously (Thangamani et al., 2015b).

Inhibition of Adhesion and Infection

The adhesion and infection experiments were performed as described previously with some modifications (Frandoloso et al., 2012; De Llano et al., 2015). In brief, MRSA ATCC35391 was exposed to GA and NGA at a concentration of 0.25 μg/mL and cultured at 37°C until the cell growth reached the logarithmic phase and a bacterial cell density of 1×10^9 CFU/mL was achieved. A prepared monolayer of keratinocytes cells (1×10^6 cells/pore) was washed with phosphate buffer saline (PBS) to eliminate antibiotics and then covered with 1 mL of GA- and NGA-treated MRSA strains. The cells were cultured at 37°C in 5% CO_2, and the unbound bacteria were removed by washing five times with PBS. One hundred fifty microliters of trypsin was added to digest and separate the cells from the adherent bacteria. In addition, 850 μL of deionized water was added and bubbled repeatedly to release the cells and the cell-associated bacteria. One hundred microliters of diluted lysis buffer was coated onto the MHA plate; the cells were cultured for 20 h; and the total number of CFUs was determined.

After the adhesion test, the unbound bacteria were removed by washing with PBS 5 times, and the infection test was conducted by adding DMEM containing gentamycin and incubating for 2 h to remove surface bacteria. The remaining procedure was the same as that for the adhesion test.

Scanning Electron Microscope

Biofilm formation was conducted as described above with glass coverslips in 24-well plates. The biofilms formed were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4°C for 10 min and then washed with PBS three times. The biofilms were then fixed with 1% osmic acid at room temperature for 10 min. Then, gradual dehydration was carried out with ethyl alcohol (60, 70, 80, 90, 95, and 100%), and tertiary butanol was used as a displacement liquid (60, 70, 80, 90, 95, and 100%). Finally, the samples were freeze-dried overnight. The specimens were then sputter coated with gold for observation using a JSM 7500 (JEOL, Tokyo, Japan).

Biofilm Assay

S. aureus ATCC 33591 was cultured in tryptic soy broth containing 1% glucose, and biofilms were formed after 24 h of incubation at 37°C. Then, the medium was removed, and the biofilms were washed with PBS. Drugs were added at concentrations of 0.25, 0.5, 1, and 2 μg/mL, and the biofilms were incubated for an additional 24 h at 37°C. The 96-well plate was washed again, and the biofilms were stained with 0.1% (wt/vol) crystal violet. Then, the 96-well plates were washed and air-dried, and finally, the biofilm mass was dissolved in 95% ethanol. A microplate reader (Bio-Tek Instruments Inc.) was used to measure the absorbance (490 nm) of the crystal violet. The data are presented as the percent biofilm mass reduction in the treated groups compared with that in the control group.

Mouse Experiments

Eight-week-old female BALB/c mice (Vital River, Beijing, China) were used in all the mouse experiments. The animal experiments were performed in accordance with animal ethics guidelines and approved protocols. The animal experiments were approved by the Animal Ethics Committee of the Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences (approval number IACUC-2018-086).

In systemic nonlethal infection, mice were intraperitoneally injected with 1.2×10^8 CFUs of *S. aureus* ATCC33591. The mice were then divided into four groups (15 mice per group) and tail vein injected with GA (5 mg/kg), NGA (5 mg/kg), vancomycin

(5 mg/kg) or vehicle (10% ethanol) alone. The mice were treated once daily for six days and euthanized after 24 h of the final administration. Organs (including heart, lung, kidney, spleen, and liver) were excised for histological analyses. Mice in the control and treated groups were subjected to the same systemic nonlethal infection protocol and submitted for histopathological examination after six days.

Skin infections were performed according to the infection model used by Purdue University with slight modification (Thangamani et al., 2015a). Briefly, mice (10 mice in each group) were injected intradermally with 4.5×10^8 CFUs of MRSA ATCC33591, and after 48 h, formation of an open wound was observed at the injection site. Then, the mice were treated with 1% GA or 1% NGA (using 20 mg of petroleum jelly as the vehicle) once a day for 9 days; the control group was treated with the vehicle alone. On the fifth day, 5 mice were selected randomly; the area around the wound was lightly swabbed with 70% ethanol; and the wound (1 cm^2) was excised, homogenized, serially diluted, and plated on MHA. The plates were incubated at 37°C for 18 h before counting the viable bacterial CFU. The remaining 5 mice in each group continued to be treated until day 9 to observe the effect of treatment on the wound.

RNA-Seq Transcriptomics

S. aureus ATCC33591 was grown to an OD600 of 0.4 from an initial value of 0.01, and GA and NGA were added to a final concentration of 1/2 × MIC. Samples were collected 1 h post treatment and preserved with RNAprotect (Qiagen, USA) following the manufacturer's instructions. The cells were pelleted by centrifugation at 5,000 × g for 10 min at 4°C. RNA was isolated using the RNeasy Mini Kit (Qiagen, USA) in accordance with the manufacturer's instructions with the following modifications: The cell pellets were homogenized in 1 mL of Tris-buffered saline (TBS) (20 mM Tris, pH 7.5) containing 0.4 mg of lysostaphin and incubated at 37°C for 15 min. Subsequently, 20 mg of lysozyme in TE buffer (20 mM Tris, pH 7.5; and 2 mM ethylene diamine tetraacetic acid, pH 7.8) was added, and the sample was incubated at 25°C for 10 min. Control samples were collected from an antibiotic-free culture, and each experiment was repeated three times.

Three independently prepared RNA samples from each strain were used for RNA-Seq. Illumina sequencing was performed by Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China) using the Illumina HiSeq2000 Truseq SBS Kit v3-HS (200 cycles) and the MiSeq Reagent Kit V2 (500 cycles/600 cycles) (Illumina Inc.). Data analyses were performed using edgeR software. Genes exhibiting 2-fold changes in expression, which were statistically significant as determined by Student's t-test ($p < 0.05$), were considered to be differentially expressed under the conditions indicated.

Real-Time RT-PCR

To verify the RNA-Seq data, we selected some genes that were downregulated and assessed the relative expression levels of these genes by real-time RT-PCR. *S. aureus* ATCC33591 cells were cultured under the same conditions as those of the RNA-Seq transcriptomics experiments. Q-RT-PCR was performed by a two-step process. These reactions were performed using an Applied Biosystems qTOWER 2.2 (Analytik Jena, Jena, Germany) real-time PCR system by using the following cycling parameters: 95°C for 5 min; 40 cycles of 95°C for 15 s, 55°C (for the cap5C gene) or 57°C for other genes for 15 s, and 72°C for 15 s; and one dissociation step of 95°C for 1 min, 55°C for 30 s, and 95°C for 30 s. All the measurements were independently conducted 3 times for 2 separate biological isolates. The sequences of all the primers used are listed in **Supplementary Table S1**.

A melting curve analysis was performed immediately after amplification to verify the specificity of the PCR amplification products. Fluorescence was measured at the end of the annealing-extension phase of each cycle. The threshold value for the fluorescence of all the samples was set manually. The reaction cycle at which the PCR product exceeded this fluorescence threshold was identified as the threshold cycle. Relative quantitation was performed by the $2-\Delta\Delta CT$ method.

Parallel Reaction Monitoring

Parallel reaction monitoring (PRM)-MS was performed by Shanghai Meiji Biology Co., Ltd. The expression levels of the proteins encoded by specific genes identified by RNA-Seq analysis were determined by quantifying the changes in the expression levels of the selected proteins before and after treatment with NGA. Specific peptide sequences were selected based on the proteins selected for PRM analysis. The chromatographic column used was a C18 column (75 μm × 25 cm; Thermo, USA) liquid chromatography was performed on an EASY-nLC 1200; the mass spectrometer used was a Q-Exactive Thermo, USA; the data acquisition software used was Thermo Xcalibur 4.0 (Thermo, USA); and Skyline software was used for quantitative analysis of the proteomics data.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). One-way ANOVA was performed between groups. For ANOVA, the observed variance is partitioned into components according to different explanatory variables. *$P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Inhibitory Activity of GA and NGA Toward MRSA

To assess the antibacterial activity of GA and NGA, 20 strains of MSSA and MRSA were selected. According to the MIC results (**Table 1**), both GA and NGA exhibited excellent inhibitory activity toward MRSA and MSSA. The MIC values for the inhibition of MSSA ranged from 0.5 μg/mL to 4 μg/mL. For MRSA inhibition, although the MIC of oxacillin was 64 μg/mL, the MICs of GA and NGA remained between 0.5 μg/mL and 4 μg/mL. Earlier reports show that the MIC of GA on MRSA strains USA3000 is 12.5 μM (Chaiyakunvat et al., 2016). In this study, we used the ATCC33591 strains and the rest of the 19 clinical strains. While the MIC of GA and NGA on all strains were between 0.5 and 4 μg/mL, significantly lower than that reported. Bacterial killing curve

TABLE 1 | MIC of GA and NGA against *Staphylococcus aureus* strains.

	MSSA				MRSA			
	Strains	**GA**	**NGA**	**Oxacillin**	**Strains**	**GA**	**NGA**	**Oxacillin**
		MIC (μg/mL)				**MIC (μg/mL)**		
Standard strain	ATCC29213	1	1	0.25	ATCC33591	1	1	128
Clinical isolates	L1	0.5	1	0.25	LN2	0.5	1	>128
	L2	0. 5	2	0.5	LN3	0.5	0.5	>128
	L4	1	2	0.25	LN4	1	0.5	64
	L5	1	1	0.25	LN6	1	1	>128
	L7	0.5	1	1	LN8	2	2	>128
	L11	4	4	0.25	LN18	1	0.5	>128
	L13	2	1	0.25	LN19	4	4	64
	L17	1	1	0.25	LN20	1	1	>128
	L22	1	0.5	0.25	LN21	2	4	>128
	L23	1	0.5	0.25	LN22	2	2	>128
	L24	1	2	1	LN23	1	1	128
	L28	2	2	0.25	LN30	2	2	>128
	L30	1	1	0.5	LN33	1	1	>128
	L31	1	1	0.25	LN36	1	1	>128
	L32	0.5	0.5	0.25	LN44	2	1	128
	L37	4	2	0.25	LN45	1	0.5	>128
	L40	1	2	0.5	LN46	1	1	>128
	L55	0.5	1	0.25	LN50	0.5	1	>128
	L56	1	0.5	0.25	LN58	0.5	0.5	>128
	L57	1	0.5	0.25	LN63	1	1	>128

and in vivo experiment results also show that the GA and NGA have very strong antibacterial activity (Chaiyakunvat et al., 2016).

Cytotoxicity

GA and NGA are extracted from the traditional Chinese medicine gamboge. It has been reported that the IC_{50} of GA on toxicity standard cell line L929 cells was 287 μg/mL, and acute injection toxicity indicated that the half lethal dose (LD_{50}) of GA was (18.59 mg/kg, 95% LD_{50}, 16.84–20.53 mg/kg) (Feng et al., 2018). GA had no significant side effects on cardiovascular, respiratory, and central nervous systems at higher doses (16 μg/kg)(Zhao et al., 2010). Although there have been many reports of strong inhibitory effects of GA and NGA on a variety of tumor cells (Pandey et al., 2016; Sun et al., 2018), while GA and NGA have also been shown to be safe for normal cells and humans.

Kinetics of Bacterial Killing

The rates of microbial killing by GA, NGA, and vancomycin were determined by exposing MRSA ATCC33591 cells to 5×MIC of each treatment over a 24-hour incubation period at 37°C. Both GA and NGA exhibited a rapid bactericidal effect, with a 3-log10 reduction (99.9% clearance) within 4 and 6 h, respectively (**Figure 2A**). In comparison, vancomycin achieved a 3-log10 bacterial reduction only after 24 h.

Compared to the control group, the GA and NGA treatment groups exhibited significant suppression of two key toxins (PVL and Hla, that injure host immune cells and promote infection of host tissues) by MRSA ATCC33591. GA and NGA exhibited better inhibitory activity toward PVL and Hla than linezolid (an antibiotic that inhibits protein synthesis) (**Figure 2B**).

Scanning electron microscopy (SEM) was utilized to observe the surface morphology of ATCC33591 before and after GA and NGA treatment. The cell walls of ATCC33591 exhibited contraction and rupture after treatment with GA and NGA, and this condition worsened with increasing concentration (**Figure 2C**). When the concentration of GA and NGA was at 2 × MIC, most of the bacteria died.

Inhibition of Adhesion and Invasion

The inhibitory effects of GA and NGA toward ATCC33591 cells adhered to keratinocytes cell were as shown in **Figure 2D**. With increasing concentration of GA and NGA, inhibition of MRSA infection increased gradually. When the concentration of GA and NGA reached 0.25 μg/mL, the adhesion rate were 69.9% and 57.6%, respectively, compared with the control group, and this difference was statistically significant ($P < 0.05$). Similar to the results observed for adhesion, the infection ability of the ATCC33591 strain treated with GA and NGA also decreased in a concentration-dependent manner (**Figure 2E**). When the concentration of GA and NGA was 0.25 μg/mL, the rates of invasion were 73.5% and 66.6% compared with the control group, respectively.

Inhibition of Biofilm Formation

Staphylococcal biofilms are intrinsically resistant to conventional antibiotics, and currently, there are no effective therapies that target microbial biofilms. Therefore, novel antibiofilm agents, treatments and strategies are needed. Since GA and NGA exhibited significant activity against planktonic bacteria, the inhibition of biofilm formation was tested.

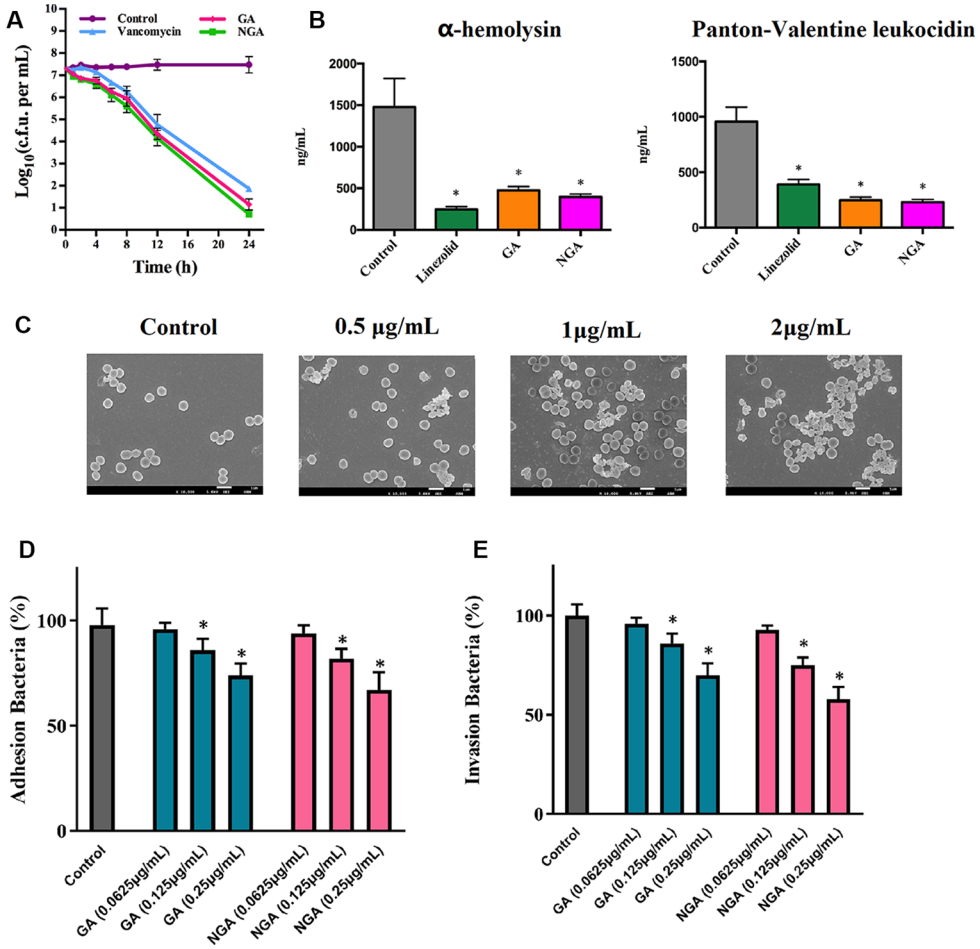

FIGURE 2 | *In vitro* antibacterial activity of gambogic acid and neogambogic acid. **(A)** Time-kill kinetics of GA and NGA against *S. aureus* ATCC33591. **(B)** Toxin production (ng/mL) in *S. aureus* ATCC33591 after treatment with GA (1μg/mL), NGA (1μg/mL) or linezolid (8μg/mL) for one hour. The results are presented as mean ± SD (n = 3). **(C)** Scanning electron microscopy images showing the structure of *S. aureus* ATCC33591 treatment with GA for one hour. Magnifications, x 10,000. **(D)** and **(E)** The adherence and invasion of *S. aureus* ATCC33591 with/without GA and NGA treatment to keratinocytes cells CM-M168. Statistical analysis was done by One-way ANOVA test between groups. P values of (*P ≤ 0.05) are considered significant.

The *in vitro* effects of GA and NGA on MRSA biofilm formation were investigated using semiquantitative crystal violet staining assays and SEM. As shown in **Figures 3A**, **B**, GA and NGA could significantly inhibit the growth of biofilms at 2 μg/mL in the crystal violet experiment, and the inhibitory effect became more apparent as the drug concentration increased. Eighty-seven percent of the biofilm formation was inhibited by GA and NGA at 8 μg/mL, and similar results were observed by SEM (**Figure 3C**). The biofilms were observed to be thick by SEM; however, after treatment for 4 h with 8 μg/mL NGA or 8 μg/mL GA, the bacterial abundance was greatly reduced, and the bacteria failed to form biofilm structures.

In Vivo Experiments

A mouse sepsis model was used to evaluate the antibacterial activity of GA and NGA *in vivo*. Mice were intraperitoneally injected with 1.2×10^8 CFUs of ATCC33591 and provided 5 mg/kg GA or NGA daily. As depicted in **Figure 4A**, treatment with GA, NGA and vancomycin led to significant reduction in the mean bacterial load in different organs. In particular, both treatments reduced the mean bacterial load by more than 1000-fold in the lungs. The histopathological inspection performed six days after infection with a nonlethal dose of MRSA ATCC 33951 revealed no changes in the heart, spleen and kidneys. While the animals exhibited moderate histopathological alterations in the lungs and liver in the control group, after treatment with GA, NGA, or vancomycin, there were no obvious histopathological alterations (**Figure 4B**).

We monitored skin necrosis in mice on days 1, 3, 5, 7 and 9 after infection with MRSA. As shown in **Figures 4C**, **D**, the areas of skin infection on the mice decreased significantly after treatment with GA and NGA, and the infection also decreased significantly. Mouse skin was collected on day 5 after inoculation, and CFU enumeration was performed; there was a clear decrease in the amount of bacteria in the GA- and NGA-treated groups (**Figure 4E**).

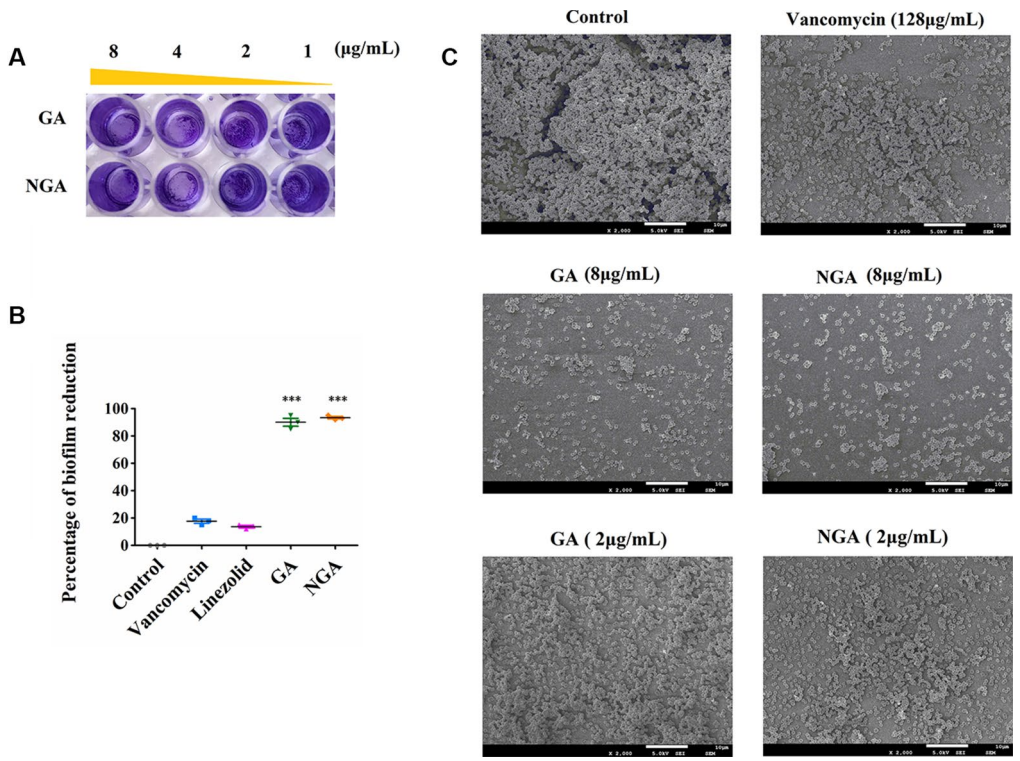

FIGURE 3 | Gambogic acid and neogambogic acid inhibit MRSA biofilm formation *in vitro*. **(A)** Crystal violet assay to assess the antibioflm activity of GA and NGA against *S. aureus* ATCC33591 bioflm. **(B)** Percent reduction of *S. aureus* ATCC33591 biofilm after treatment with GA and NGA (8μg/mL). **(C)** Scanning electron microscopy images showing the structure of *S. aureus* ATCC33591 biofilm. Magnifications, x 2,000. P values of (***P ≤ 0.005) are considered significant.

Transcriptomics

To determine how GA and NGA inhibit MRSA, transcriptomic studies were conducted. We compared the transcriptome of untreated ATCC33591 with those of the strain treated with GA and NGA. A total of 2,944 genes were detected; 149 and 178 genes were differentially expressewd in the GA- and NGA-treated groups compared with the control group, with 74 and 102 downregulated genes, respectively, and 75 and 76 upregulated genes, respectively. These differentially expressed genes were selected based on logFC values greater than 2 and $p < 0.05$. The GA and NGA data were the results of the interaction of each of these compounds with ATCC33591 and were similar because of the similar structures of GA and NGA. Except for the slight numerical difference between the two sets of data, most of the genetic change trends were consistent, which suggested the accuracy and reliability of the data.

To better understand the functions of these differentially expressed genes, we conducted GO and KEGG distribution analyses. The differentially expressed genes were divided into three GO categories (**Figure 5A**) – cellular component, biological process, and molecular function – according to sequence homology. GO categories shows that the gene expression trends of the MRSA strains were quite similar after GA and NGA treatment. In terms of functional classification, genes associated with biological adhesion, cell killing, multiorganism process, negative regulation of biological process, and reproduction were significantly downregulated, while both biological adhesion and cell killing were key factors associated with biofilm formation. This result is consistent with our previous observations. In terms of cellular composition, the downregulated genes were mainly distributed in the extracellular region, and the downregulation was caused by the inhibition of some related virulence factors. Analysis of the molecular function showed that some activities were inhibited, such as signal transduction activity, protein-binding transcription factor activity, and receptor activity. The down regulated KEGG pathway analysis showed that the differentially expressed genes were mainly clustered in the ABC transporters, *Staphylococcus aureus* infection, and two-component system categories (**Figure 5B**).

Simultaneously, we further analyzed 102 downregulated genes. We found that there was significant downregulation of many genes associated with virulence factors, two-component regulatory systems, cell wall synthesis and several energy metabolism related genes, which was shown in **Table 2**.

The *in vitro* experiments showed that GA and NGA could effectively inhibit the growth, infection, adhesion, exotoxin secretion, and biofilm formation of MRSA, which was consistent with the key pathways identified in the GO and KEGG analyses.

Virulence Factors and Two-Component Systems

S. aureus is a pathogen that causes many diseases, including pneumonia, septicemia, and meningitis, which are caused

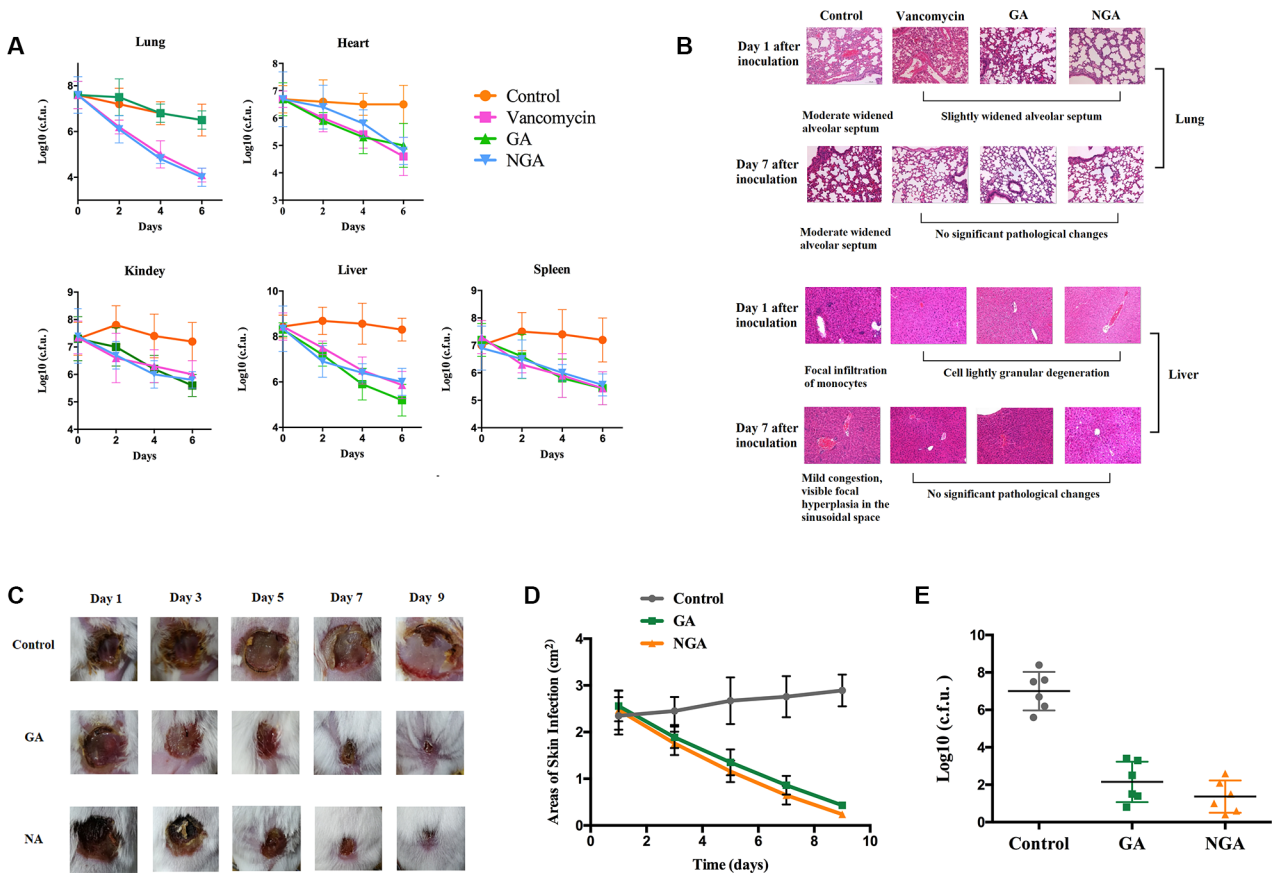

FIGURE 4 | GA and NGA can effectively inhibit septicemic and skin infection caused by *S. aureus* ATCC33591 *in vivo*. **(A)** Fifteen mice per group were infected (i.p) with non-lethal dose of *S. aureus* ATCC33591 and treated orally with GA, NGA, vancomycin (5 mg/kg) or the vehicle alone for six days (one dose per day). 24 h after the last treatment, mice were euthanized and their organs were excised and homogenized in TSB to count viable MRSA colonies. The number of CFU from each mouse is plotted as individual points. Values are the mean of triplicate results with standard deviation bars. **(B)** Histological evaluation of lung and liver of mice infected with *S. aureus* ATCC33591 receiving no treatment or a treatment with GA and NGA. Both lung and liver in control group demonstrated acute inflammation, in the treated, group no apparent pathological changes were observed. **(C)** Ten mice per group with subcutaneous infection *S. aureus* ATCC33591. After the wound is formed the mice were treated with 1% GA or 1% NGA once a day for 9 d. Compared with the control group, the wounds healed well after GA and NGA treatment, the wound area **(D)** and the amount of bacteria **(E)** were significantly reduced.

by multiple virulence factors produced by this bacterium. These virulence factors are regulated by two-component systems (such as *agr*, *srrAB*, *arlRS*, *vraSR*, and *saeRS*) (Canovas et al., 2016).

The *saeR* and *saeS* genes were downregulated 3.53 and 4.32 times, respectively, after treatment with GA and 9.50 and 10.62 times, respectively, after treatment with NGA. Among the 16 TCS systems of *S. aureus*, *saeRS* play an important role in regulating more than 20 important virulence factors, such as hemolysins, leukocidins, coagulases and immune evasion molecules (Liu et al., 2016).

In our RNA-Seq results, we found that the expression levels of the following 16 genes associated with virulence factors were downregulated observably: the hemolysin-related genes *splABCDF* (*Spl* is involved in host colonization and infection and is considered to be a potential drug target) (Paharik et al., 2016); the IgG-binding protein related gene *sbi* (which can help bacteria escape macrophage phagocytosis and neutrophil killing) (Zhao et al., 2016); the delta-hemolysin gene *hld*; the gamma-hemolysin and leukocytotoxin-related genes *SAV2004*, *hlg*ABC and *Luk*DE; and three genes *SAV1155*, *SAV1158* and *SAV1159* which associated with fibrinogen-binding proteins. In addition, these virulence factors are capable of directly interacting with proteins in the *saeRS* two-component system. STRING network analysis was used to examine the relationships among the proteins whose expression decreased more than 4 times after GA and NGA treatment. A distinct network of *saeRS*-centric protein interactions was constructed and is shown in **Figure 6**. According to the results of the interaction analysis, *saeRS*-centered virulence factors and energy-metabolism-related proteins were significantly downregulated after treatment. Previous research has shown that the structural analogues of GA and NGA can inhibit *S. aureus* invasion of cells, the results of this study are consistent with our findings (Chaiyakunvat et al., 2016). By inhibiting the *saeRS* two-component system of MRSA strain, the expression of virulence factors of the strain

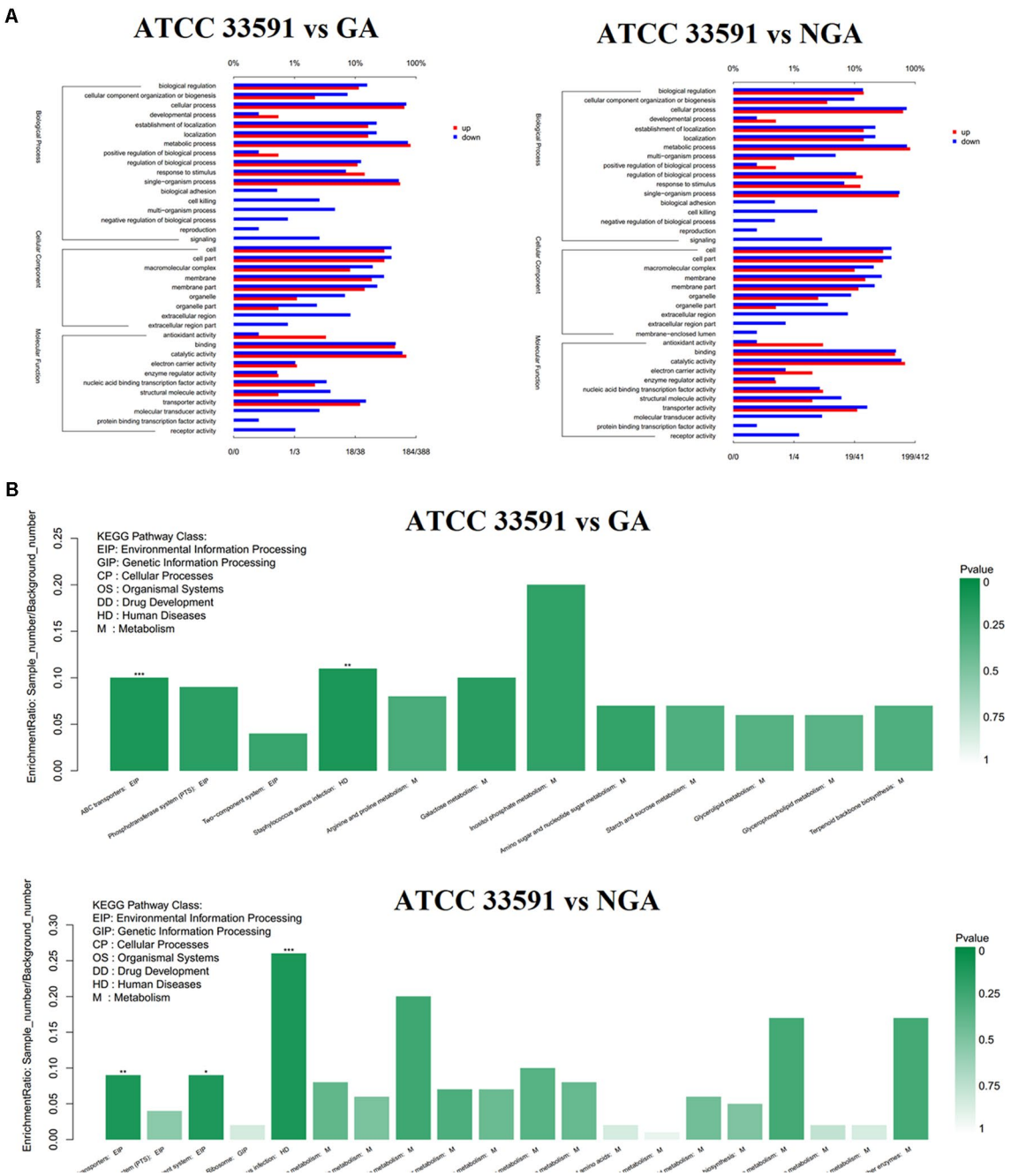

FIGURE 5 | RNA-Seq gene expression results for *S. aureus* ATCC33591 cells treated and not treated with GA and NGA. **(A)** GO enrichment analysis of differently expressed genes. **(B)** Down-regulated genes enriched in the KEGG Pathway.

was inhibited by the gambogic acid and neogambogic acid, thus inhibiting the invasion of MRSA strain to the host, which may be the main mechanism of GA and NGA antibacterial action.

In addition to virulence, both *agr* and *saeRS* influence biofilm formation in *S. aureus*, with *agr* acting *via* the production of phenol-soluble modulins (PSMs) (Surewaard et al., 2013) and *saeRS* by repressing the production of extracellular proteases that degrade proteins important for biofilm formation (Boles and Horswill, 2008).

Several compounds have been found to inhibit the expression of virulence-related genes in *S. aureus* by inhibiting the *agrAC* two-component system. Norlichexanthone has been shown to inhibit biofilm formation by inhibiting *agrAC* and *saeRS* expression. Some studies have shown that increased expression of the quorum sensing system can effectively inhibit biofilm formation (Baldry et al., 2016). Although *agrAC* and *saeRS* were inhibited after treatment with drugs such as GA, NGA, and norlichexanthone,

TABLE 2 | Key genes of ATCC33591 down-regulated by GA and NGA.

Gene ID	Gene name	Description	Log2 flod change (GA)	Log2 flod change (NGA)
SAV0023	None	5'-nucleotidase	–2.13	–2.16
SAV0095	plc	1-phosphatidylinositol phosphodiesterase	–2.67	–3.49
SAV0104	None	Na/Pi cotransporter	–2.28	–2.38
SAV0191	None	N-acetylmuramic acid-6-phosphate etherase	–2.9	–3.42
SAV0192	None	PTS system EIIBC component	–2.5	–2.82
SAV0193	None	RpiR family transcriptional regulator	–2.25	–2.56
SAV0216	None	arabinogalactan ABC transporter permease	–1.91	–2.17
SAV0217	None	oxidoreductase	–1.86	–2.39
SAV0218	None	NADH-dependent dehydrogenase	–1.86	–2.34
SAV0219	None	xylose isomerase	–1.58	–2.16
SAV0222	uhpT	antiporter [Staphylococcus sugar phosphate antiporter	–1.68	–2.16
SAV0259	scdA	Iron-sulfur cluster repair protein ScdA	–1.81	–2.05
SAV0261	lytR	LytR family transcriptional regulator	–1.91	–2.18
SAV0285	None	type VII secretion protein EsaB	–2.56	–2.51
SAV0315	nanA	N-acetylneuraminate lyase	–1.75	–2.25
SAV0320	geh	lipase	–2.81	–3.84
SAV0432	hsdS	restriction endonuclease subunit S	–1.98	–2.32
SAV0450	None	cobalamin synthesis protein CobW	–2.26	–2.82
SAV0458	None	sodium-dependent transporter	–1.31	–2.13
SAV0465	None	peptidase M23B	–3.07	–2.63
SAV0539	rplJ	50S ribosomal protein L10	–1.39	–2
SAV0631	None	manganese ABC transporter substrate-binding protein	–2.14	–2.16
SAV0632	None	membrane protein ABC transporter permease	–1.96	–2.14
SAV0633	None	phosphonate ABC transporter ATP-binding protein	–1.9	–2.09
SAV0705	saeS	histidine protein kinase	–2.9	–3.41
SAV0706	saeR	response regulator saeR	–1.82	–3.25
SAV0815	nuc	nuclease	–2.47	–3.07
SAV1052	truncated-atl	mannosyl-glycoprotein endo-beta-N-acetylglucosamidase	–2.72	–2.29
SAV1131	None	heme transporter IsdC	–1.58	–2.1
SAV1135	None	sortase B	–3.56	–2.71
SAV1136	None	heme-degrading monooxygenase IsdG, partial	–2.59	–3.96
SAV1155	None	fibrinogen-binding protein	–1.98	–3.14
SAV1158	None	fibrinogen-binding protein	–2.46	–2.85
SAV1159	None	fibrinogen-binding protein	–1.82	–2.12
SAV1163	None	alpha-hemolysin	–2.79	–4.02
SAV1169	argF	ornithine carbamoyltransferase	–1.85	–2.08
SAV1436	None	quinolone resistance protein NorB	–1.66	–2.05
SAV1437	None	amino acid permease	–1.62	–2.49
SAV1550	None	5-formyltetrahydrofolate cyclo-ligase	–2.09	–2.15
SAV1660	truncated-radC	hypothetical protein	–2.2	–2.6
SAV1661	None	type III leader peptidase	–1.84	–2.1
SAV1686	None	NrdR family transcriptional regulator	–1.79	–2.02
SAV1709	ald	alanine dehydrogenase	–1.72	–2.01
SAV1799	None	calcium-binding protein	–1.95	–2.27
SAV1809	splF	serine protease	–2.6	–4.04
SAV1810	splD	serine protease	–2.63	–4.02
SAV1811	splC	serine protease	–2.59	–3.87
SAV1812	splB	serine protease	–2.72	–4.2
SAV1813	splA	serine protease	–2.64	–4.18
SAV1819	lukD	gamma-hemolysin subunit B	–2.32	–3.44
SAV1820	lukE	gamma-hemolysin subunit A	–2.41	–3.05
SAV1909	None	cysteine protease	–2.21	–2.33
SAV1910	None	staphostatin A	–2.4	–2.05
SAV1914	None	Nitric-oxide synthase	–2.11	–2.18
SAV1937	None	extracellular adherence protein Eap/Map	–1.57	–2.48
SAV1938	None	protein map	–1.74	–2.24
SAV1942	None	inhibitor	–2.02	–3.32
SAV2004	None	gamma-hemolysin subunit B	–2.11	–3.37
SAV2005	None	succinyl-diaminopimelate desuccinylase	–1.64	–3.21
SAV2035	hld	delta-hemolysin	–1.87	–2.24
SAV2038	agrC	histidine kinase	–1.94	–2.09

(Continued)

TABLE 2 | Continued

Gene ID	Gene name	Description	Log2 flod change (GA)	Log2 flod change (NGA)
SAV2039	agrA	histidine kinase	–2.09	–2.34
SAV2117	None	N5-glutamine S-adenosyl-L-methionine-dependent methyltransferase	–2.18	–2.11
SAV2119	tdk	thymidine kinase	–.94	–2.28
SAV2177	None	iron citrate ABC transporter substrate-binding protein	–2.09	–2.09
SAV2304	None	secretory antigen SsaA, partial	–3.37	–2.98
SAV2363	None	LytTR family transcriptional regulator	–1.94	–2.03
SAV2418	sbi	hypothetical protein	–2.04	–4.03
SAV2419	hlgA	gamma-hemolysin subunit A	–1.44	–3.81
SAV2420	hlgC	Gamma-hemolysin C subunit HlgC	–1.12	–3.55
SAV2421	hlgB	gamma-hemolysin subunit B	–1.24	–3.45
SAV2463	None	peptide ABC transporter ATP-binding protein	–2.21	–2.53
SAV2464	None	peptide ABC transporter ATP-binding protein	–2.68	–2.45
SAV2465	None	peptide ABC transporter permease	–1.99	–2.01
SAV2470	None	diaminopimelate epimerase	–1.55	–2.04
SAV2514	None	Probable transport protein	–2.05	–2.1
SAV2544	None	peptidase M23B	–2.89	–2.41
SAV2569	isaA	transglycosylase	–2.68	–2.39
SAV2632	arcC	carbamate kinase	–1.83	–2.18
SAV2634	arcB	ornithine carbamoyltransferase	–1.71	–2.15
SAV2662	None	capsular polysaccharide biosynthesis protein Cap8C	–2.9	–2.76
SAV2663	None	capsular polysaccharide biosynthesis protein Cap5B	–2.58	–2.69

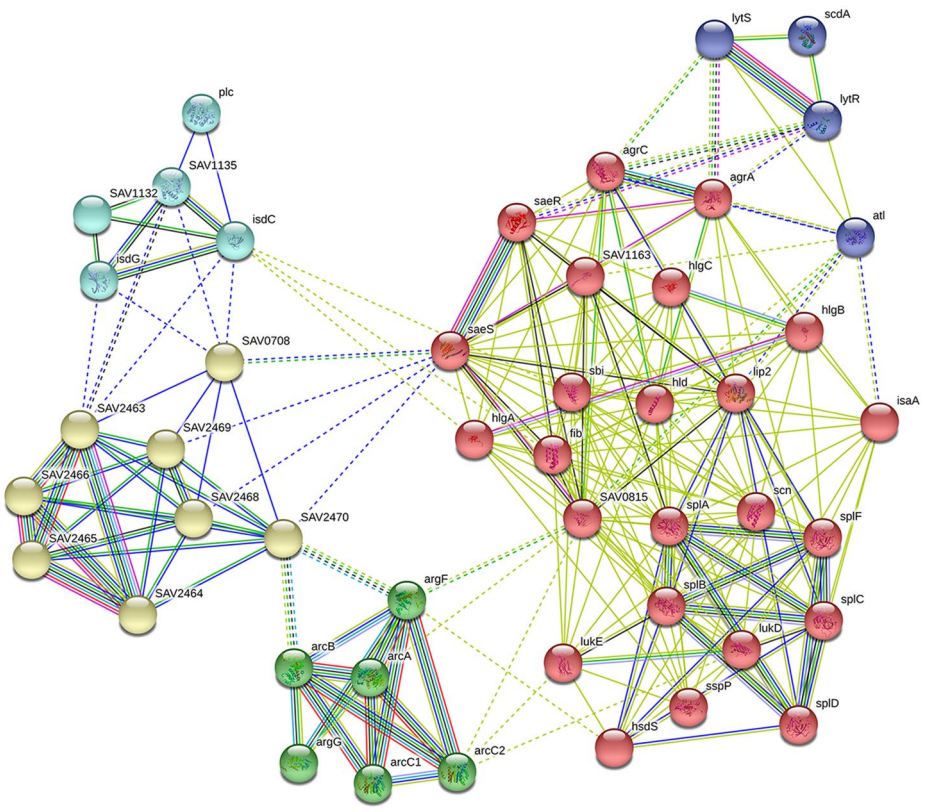

FIGURE 6 | Gene interaction network including down-regulated expressed genes of *S. aureus* ATCC33591 cells treated with GA and NGA after using STRING bioinformatic tool.

the consequent downregulation of virulence factors may be the key to the inhibition of biofilm formation.

Cell Wall Formation

The significant direct inhibitory effects of GA and NGA on MRSA and the distinct shrinkage and rupture of the cell walls of the bacteria observed by SEM indicate that these compounds inhibit cell wall synthesis.

According to the transcriptomic analysis, expression of some of the key genes associated with cell wall formation was significantly inhibited, with inhibition ratios greater than 4. Capsular polysaccharides are important components of the cell wall. SAV2662 and SAV2663 are two capsular polysaccharide synthesis proteins that were significantly downregulated after treatment with GA and NGA.

In addition, after GA and NGA treatment, SAV0465 (N-acetylmuramoyl-L-alanine amidase), SAV0192 (N-acetylmuramic acid 6-phosphate etherase) and SAV0192 (phosphatase system sucrose-specific IIBC component) were significantly downregulated. Peptidoglycan forms an envelope structure in which bacteria maintain their morphology. This structure is formed by the crosslinking of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) by short peptides. PGN encases the bacterial cell, forming a large, net-like, turgor-resisting and shape-maintaining envelope structure that is composed of glycan strands of two alternating β-1,4-linked sugars, GlcNAc and MurNAc, crosslinked by short peptides (Gutierrez et al., 2018).

MurNAc-6p is the product of MurNAc uptake and phosphorylation of MurNAc by the specific PTS transporter MurP, which plays an important role in the formation of peptide polysaccharides. SAV0191 and SAV0192 are the transcriptional regulators of MurNAc-6p in *S. aureus*. The results indicate that GA and NGA inhibit the cell wall formation in MRSA by inhibiting the synthesis of MurNAc, which could be an important drug target. In addition, *lip2* (a glycerol ester hydrolase) and SAV0631 (a lipoprotein) are two proteins related to cell wall formation that were also inhibited.

Q-RT-PCR and PRM

Based on RNA-Seq results, we hypothesized that GA and NGA could regulate virulence factors and other proteins directly associated with *saeRS* by inhibiting the expression of the *saeRS* proteins. To validate our hypothesis, Q-RT-PCR and PRM were conducted. Q-RT-PCR could accurately reflect the changes in MRSA gene expression before and after GA and NGA treatment. Due to the high specificity and sensitivity of PRM, this method has been widely used for the determination of target protein content.

Twenty differentially expressed proteins with distinct changes in expression were selected for Q-RT-PCR, and the detection results are shown in **Figure 7**. The variation trend for differential gene expression observed by Q-RT-PCR was consistent with that observed by RNA-Seq. Expression of 7 genes encoding the *saeRS*, *agrAC* and *sbi* proteins was detected before and after GA and NGA treatment by PRM. The results are shown in **Table 3**, we found that the expression of the proteins encoded by *saeRS* and *agrC* were get reduced after treatment with GA and NGA, which confirmed our hypothesis. However the differences in the expression of the other proteins were not significant.

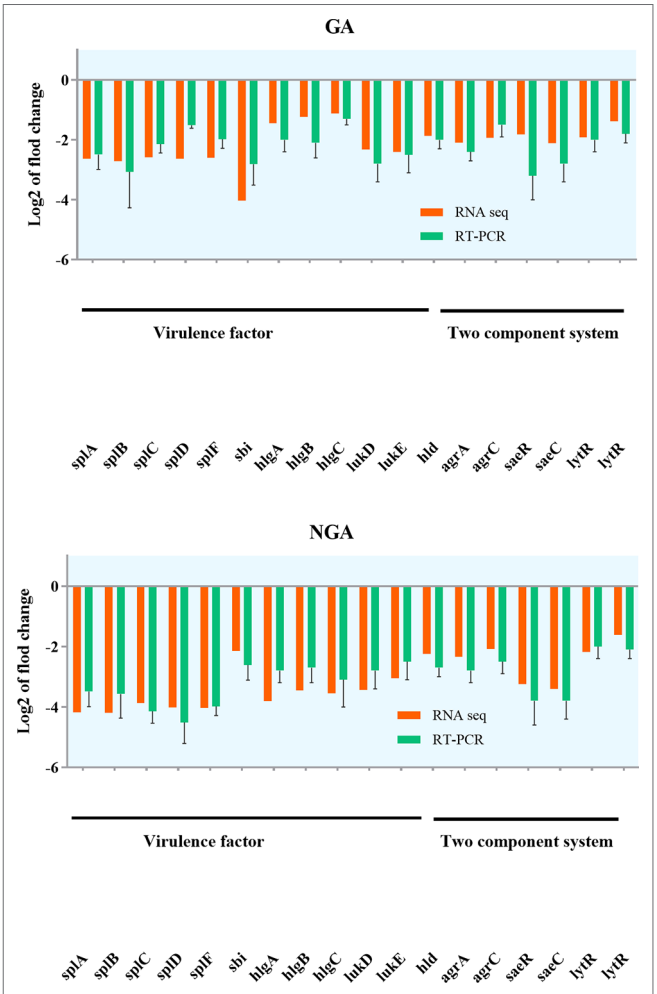

FIGURE 7 | Validation of RNA-seq data for selected genes by real-time PCR.

TABLE 3 | Quantity of protein expression based on the PRM detection.

Protein name	Gene name	Peptide sequence	Ration	
			GA vs. control	NGA vs. control
Response regulator SaeR	*saeR*	LDIPFIYLTAK	0.79	0.63
Histidine protein kinase SaeS	*saeS*	ILTNLLDNALK	0.86	0.61
Accessory gene regulator A	*agrA*	ELSQLDDR	0.81	1.27
Accessory gene regulator C	*agrC*	GLGLSTLK	0.82	0.65
Immunoglobulin-binding protein	*sbi*	GAIDQTVLTVLGSGSK	0.93	1.11
Gamma-hemolysin component C	*hlgC*	GSSDTSEFEITYGR	1.36	2.05
Delta-hemolysin	*hld*	WIIDTVNK	1.26	1.95

The results of this study suggested that both GA and NGA have significant anti-MRSA activity *in vivo* and *in vitro*, especially in the inhibition of biofilm formation and skin infection by MRSA. Transcriptome sequencing, RT-PCR and PRM were performed to elucidate the pathway *via* which GA and NGA downregulate the expression of the *saeRS*, a two-component system in MRSA, thus affecting the generation of virulence factors and biofilms by MRSA. In addition, GA and NGA also inhibited cell wall formation in the MRSA strains.

CONCLUSION

This study reported the anti-MRSA activity of GA and NGA, including anti-biofilm formation activity *in vivo* and *in vitro*. GA and NGA were found to exert antibacterial activity by inhibiting the bacterial *saeRS* two-component system, providing new evidence for the development of anti-bacterial drugs. GA and NGA are cytotoxic but high sensitive and effective to MRSA, furthermore toxicity can be reduced by modification of the chemical structures of these compounds. Hence, we are confident that these compounds have potential applications as anti-MRSA drugs.

DATA AVAILABILITY

All the sequencing reads have been submitted to the NCBI short-read archive (SRA) with accession number SAMN10230086.

ETHICS STATEMENT

The animal experiment was approved by the Animal Ethics Committee of Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences.

AUTHOR CONTRIBUTIONS

XH and SL designed research. XH, YJ and QY analyzed data. XH, WZ, QY, ZD and YJ performed research. XH wrote the paper.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Alam, M. T., Read, T. D., Petit, R. A., 3rd, Boyle-Vavra, S., Miller, L. G., Eells, S. J., et al. (2015). Transmission and microevolution of USA300 MRSA in U.S. households: evidence from whole-genome sequencing. *MBio* 6, e00054. doi: 10.1128/mBio.00054-15

Baldry, M., Nielsen, A., Bojer, M. S., Zhao, Y., Friberg, C., Ifrah, D., et al. (2016). Norlichexanthone reduces virulence gene expression and biofilm formation in staphylococcus aureus. *PLoS One* 11, e0168305. doi: 10.1371/journal.pone.0168305

Boles, B. R., and Horswill, A. R. (2008). Agr-mediated dispersal of *Staphylococcus aureus* biofilms. *PLoS Pathog.* 4, e1000052. doi: 10.1371/journal.ppat.1000052

Boyle-Vavra, S., Yin, S., and Daum, R. S. (2006). The VraS/VraR two-component regulatory system required for oxacillin resistance in community-acquired methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 262, 163–171. doi: 10.1111/j.1574-6968.2006.00384.x

Canovas, J., Baldry, M., Bojer, M. S., Andersen, P. S., Grzeskowiak, P. K., Stegger, M., et al. (2016). Cross-talk between *Staphylococcus aureus* and other staphylococcal species *via* the agr quorum sensing system. *Front. Microbiol.* 7, 1733. doi: 10.3389/fmicb.2016.01733

Chaiyakunvat, P., Anantachoke, N., Reutrakul, V., and Jiarpinitnun, C. (2016). Caged xanthones: potent inhibitors of global predominant MRSA USA300. *Bioorg. Med. Chem. Lett.* 26, 2980–2983. doi: 10.1016/j.bmcl.2016.05.030

Chen, F., Zhang, X. H., Hu, X. D., Zhang, W., Lou, Z. C., Xie, L. H., et al. (2015). Enhancement of radiotherapy by ceria nanoparticles modified with neogambogic acid in breast cancer cells. *Int. J. Nanomed.* 10, 4957–4969. doi: 10.2147/IJN.S82980

Cho, H., Jeong, D. W., Liu, Q., Yeo, W. S., Vogl, T., Skaar, E. P., et al. (2015). Calprotectin increases the activity of the *SaeRS* two component system and murine mortality during *Staphylococcus aureus* infections. *PLoS Pathog.* 11, e1005026. doi: 10.1371/journal.ppat.1005026

David, M. Z., and Daum, R. S. (2017). Treatment of *Staphylococcus aureus* infections. *Curr. Top Microbiol. Immunol.* 409, 325–383. doi: 10.1007/82_2017_42

De Llano, D. G., Esteban-Fernandez, A., Sanchez-Patan, F., Martin-Alvarez, P. J., Moreno-Arribas, M. V., and Bartolome, B. (2015). Anti-adhesive activity of cranberry phenolic compounds and their microbial-derived metabolites against uropathogenic *Escherichia coli* in bladder epithelial cell cultures. *Int. J. Mol. Sci.* 16, 12119–12130. doi: 10.3390/ijms160612119

Den Reijer, P. M., Haisma, E. M., Lemmens-Den Toom, N. A., Willemse, J., Koning, R. I., Demmers, J. A., et al. (2016). Correction: detection of alpha-toxin and other virulence factors in biofilms of *Staphylococcus aureus* on polystyrene and a human epidermal model. *PLoS One* 11, e0152544. doi: 10.1371/journal.pone.0152544

Federman, C., Ma, C., and Biswas, D. (2016). Major components of orange oil inhibit *Staphylococcus aureus* growth and biofilm formation, and alter its virulence factors. *J. Med. Microbiol.* 65, 688–695. doi: 10.1099/jmm.0.000286

Feng, Z., Wang, Z., Yang, Y., Du, Y., Cui, S., Zhang, Y., et al. (2018). Development of a safety and efficacy nanoemulsion delivery system encapsulated gambogic acid for acute myeloid leukemia *in vitro* and *in vivo*. *Eur. J. Pharm. Sci.* 125, 172–180. doi: 10.1016/j.ejps.2018.10.001

Ferro, T. A., Araujo, J. M., Dos Santos Pinto, B. L., Dos Santos, J. S., Souza, E. B., Da Silva, B. L., et al. (2016). Cinnamaldehyde inhibits *Staphylococcus aureus* virulence factors and protects against infection in a galleria mellonella model. *Front. Microbiol.* 7, 2052. doi: 10.3389/fmicb.2016.02052

Fournier, B., and Hooper, D. C. (2000). A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of *Staphylococcus aureus*. *J. Bacteriol.* 182, 3955–3964. doi: 10.1128/JB.182.14.3955-3964.2000

Frandoloso, R., Martinez-Martinez, S., Gutierrez-Martin, C. B., and Rodriguez-Ferri, E. F. (2012). Haemophilus parasuis serovar 5 Nagasaki strain adheres and invades PK-15 cells. *Vet. Microbiol.* 154, 347–352. doi: 10.1016/j.vetmic.2011.07.022

Giraudo, A. T., Calzolari, A., Cataldi, A. A., Bogni, C., and Nagel, R. (1999). Corrigendum to the sae locus of *Staphylococcus aureus* encodes a two-component

regulatory system. *FEMS Microbiol. Lett.* 180, 117. doi: 10.1111/j.1574-6968.1999.tb08785.x

Guo, H., Hall, J. W., Yang, J., and Ji, Y. (2017). The *SaeRS* two-component system controls survival of *Staphylococcus aureus* in human blood through regulation of coagulase. *Front. Cell Infect. Microbiol.* 7, 204. doi: 10.3389/fcimb.2017.00204

Gutierrez, D., Fernandez, L., Rodriguez, A., and Garcia, P. (2018). Are phage lytic proteins the secret weapon to kill *Staphylococcus aureus*? *MBio* 9 (1), e01923-17. doi: 10.1128/mBio.01923-17

Hall, J. W., Yang, J., Guo, H., and Ji, Y. (2017). The *Staphylococcus aureus* AirSR two-component system mediates reactive oxygen species resistance *via* transcriptional regulation of staphyloxanthin production. *Infect. Immun.* 85 (2), e00838-16. doi: 10.1128/IAI.00838-16

Hua, X., Yang, Q., Zhang, W., Dong, Z., Yu, S., Schwarz, S., et al. (2018). Antibacterial activity and mechanism of action of aspidinol against multi-drug-resistant methicillin-resistant *Staphylococcus aureus*. *Front. Pharmacol.* 9, 619. doi: 10.3389/fphar.2018.00619

Infectious Diseases Society Of America, Spellberg, B., Blaser, M., Guidos, R. J., Boucher, H. W., Bradley, J. S., et al. (2011). Combating antimicrobial resistance: policy recommendations to save lives. *Clin. Infect. Dis.* 52 (Suppl 5), S397–S428. doi: 10.1093/cid/cir153

Jin, G., Wang, F. F., Li, T., Jia, D. D., Shen, Y., and Xu, H. C. (2018). Neogambogic acid suppresses receptor activator of nuclear factor kappab ligand (RANKL)-induced osteoclastogenesis by inhibiting the jnk and nf-kappab pathways in mouse bone marrow-derived monocyte/macrophages. *Med. Sci. Monit.* 24, 2569–2577. doi: 10.12659/MSM.909651

Lehar, S. M., Pillow, T., Xu, M., Staben, L., Kajihara, K. K., Vandlen, R., et al. (2015). Novel antibody-antibiotic conjugate eliminates intracellular *S. aureus*. *Nature* 527, 323–328. doi: 10.1038/nature16057

Liu, Q., Yeo, W. S., and Bae, T. (2016). The *SaeRS* two-component system of *Staphylococcus aureus*. *Genes (Basel)* 7 (10), 81. doi: 10.3390/genes7100081

Miyazaki, S., Matsumoto, Y., Sekimizu, K., and Kaito, C. (2012). Evaluation of *Staphylococcus aureus* virulence factors using a silkworm model. *FEMS Microbiol. Lett.* 326, 116–124. doi: 10.1111/j.1574-6968.2011.02439.x

Mohammad, H., Thangamani, S., and Seleem, M. N. (2015). Antimicrobial peptides and peptidomimetics - potent therapeutic allies for staphylococcal infections. *Curr. Pharm. Des.* 21, 2073–2088. doi: 10.2174/1381612821666150310102702

Ohadian Moghadam, S., Pourmand, M. R., and Aminharati, F. (2014). Biofilm formation and antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* isolated from burn patients, Iran. *J. Infect. Dev. Ctries.* 8, 1511–1517. doi: 10.3855/jidc.5514

Paharik, A. E., Salgado-Pabon, W., Meyerholz, D. K., White, M. J., Schlievert, P. M., and Horswill, A. R. (2016). The spl serine proteases modulate *Staphylococcus aureus* protein production and virulence in a rabbit model of pneumonia. *mSphere* 1 (5), e00208-16. doi: 10.1128/mSphere.00208-16

Pandey, M. K., Karelia, D., and Amin, S. G. (2016). Gambogic acid and its role in chronic diseases. *Adv. Exp. Med. Biol.* 928, 375–395. doi: 10.1007/978-3-319-41334-1_15

Pozzi, C., Waters, E. M., Rudkin, J. K., Schaeffer, C. R., Lohan, A. J., Tong, P., et al. (2012). Methicillin resistance alters the biofilm phenotype and attenuates virulence in *Staphylococcus aureus* device-associated infections. *PLoS Pathog.* 8, e1002626. doi: 10.1371/journal.ppat.1002626

Smith, K., Gould, K. A., Ramage, G., Gemmell, C. G., Hinds, J., and Lang, S. (2010). Influence of tigecycline on expression of virulence factors in biofilm-associated cells of methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 54, 380–387. doi: 10.1128/AAC.00155-09

Sun, R., Zhang, H. M., and Chen, B. A. (2018). Anticancer activity and underlying mechanism of neogambogic acid. *Chin. J. Nat. Med.* 16, 641–643. doi: 10.1016/S1875-5364(18)30103-1

Surewaard, B. G., De Haas, C. J., Vervoort, F., Rigby, K. M., Deleo, F. R., Otto, M., et al. (2013). Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. *Cell Microbiol.* 15, 1427–1437. doi: 10.1111/cmi.12130

Tavares, A., Nielsen, J. B., Boye, K., Rohde, S., Paulo, A. C., Westh, H., et al. (2014). Insights into alpha-hemolysin (Hla) evolution and expression among *Staphylococcus aureus* clones with hospital and community origin. *PLoS One* 9, e98634. doi: 10.1371/journal.pone.0098634

Tenover, F. C., and Goering, R. V. (2009). Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. *J. Antimicrob. Chemother.* 64, 441–446. doi: 10.1093/jac/dkp241

Thangamani, S., Mohammad, H., Abushahba, M. F., Hamed, M. I., Sobreira, T. J., Hedrick, V. E., et al. (2015a). Exploring simvastatin, an antihyperlipidemic drug, as a potential topical antibacterial agent. *Sci. Rep.* 5, 16407. doi: 10.1038/srep16407

Thangamani, S., Younis, W., and Seleem, M. N. (2015b). Repurposing ebselen for treatment of multidrug-resistant staphylococcal infections. *Sci. Rep.* 5, 11596. doi: 10.1038/srep11596

Udo, E. E., and Al-Sweih, N. (2017). Dominance of community-associated methicillin-resistant *Staphylococcus aureus* clones in a maternity hospital. *PLoS One* 12, e0179563. doi: 10.1371/journal.pone.0179563

Vazquez-Sanchez, D., Galvao, J. A., and Oetterer, M. (2018). Contamination sources, biofilm-forming ability and biocide resistance of *Staphylococcus aureus* in tilapia-processing facilities. *Food Sci. Technol. Int.* 24, 209–222. doi: 10.1177/1082013217742753

Wang, K., Tang, Y., Sun, M., Lu, B., Zhu, H., Ji, O., et al. (2011). The mechanism of neogambogic acid-induced apoptosis in human MCF-7 cells. *Acta Biochim. Biophys. Sin. (Shanghai)* 43, 698–702. doi: 10.1093/abbs/gmr063

Zhang, W., Zhang, M., Wang, Z., Cheng, Y., Liu, H., Zhou, Z., et al. (2016). Neogambogic acid prevents silica-induced fibrosis *via* inhibition of high-mobility group box 1 and MCP-1-induced protein 1. *Toxicol. Appl. Pharmacol.* 309, 129–140. doi: 10.1016/j.taap.2016.09.003

Zhao, F., Chong, A. S., and Montgomery, C. P. (2016). Importance of B Lymphocytes and the IgG-binding protein Sbi in *Staphylococcus aureus* skin infection. *Pathogens* 5 (1), 12. doi: 10.3390/pathogens5010012

Zhao, L., Zhen, C., Wu, Z., Hu, R., Zhou, C., and Guo, Q. (2010). General pharmacological properties, developmental toxicity, and analgesic activity of gambogic acid, a novel natural anticancer agent. *Drug Chem. Toxicol.* 33, 88–96. doi: 10.3109/01480540903173534

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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Pharmacotherapy of Lower Respiratory Tract Infections in Elderly—Focused on Antibiotics

*Yang Liu[1,2], Yan Zhang[1,2], Wanyu Zhao[1,2], Xiaolei Liu[1,2], Fengjuan Hu[1,2] and Birong Dong[1,2]**

[1] The Center of Gerontology and Geriatrics, West China Hospital, Sichuan University, Chengdu, China, [2] Geriatric Health Care and Medical Research Center, Sichuan University, Chengdu, China

Lower respiratory tract infections (LRTIs) refer to the inflammation of the trachea, bronchi, bronchioles, and lung tissue. Old people have an increased risk of developing LRTIs compared to young adults. The prevalence of LRTIs in the elderly population is not only related to underlying diseases and aging itself, but also to a variety of clinical issues, such as history of hospitalization, previous antibacterial therapy, mechanical ventilation, antibiotic resistance. These factors mentioned above have led to an increase in the prevalence and mortality of LRTIs in the elderly, and new medical strategies targeting LRTIs in this population are urgently needed. After a systematic review of the current randomized controlled trials and related studies, we recommend novel pharmacotherapies that demonstrate advantages for the management of LRTIs in people over the age of 65. We also briefly reviewed current medications for respiratory communicable diseases in the elderly. Various sources of information were used to ensure all relevant studies were included. We searched Pubmed, MEDLINE (OvidSP), EMBASE (OvidSP), and ClinicalTrials.gov. Strengths and limitations of these drugs were evaluated based on whether they have novelty of mechanism, favorable pharmacokinetic/pharmacodynamic profiles, avoidance of interactions and intolerance, simplicity of dosing, and their ability to cope with challenges which was mainly evaluated by the primary and secondary endpoints. The purpose of this review is to recommend the most promising antibiotics for treatment of LRTIs in the elderly (both in hospital and in the outpatient setting) based on the existing results of clinical studies with the novel antibiotics, and to briefly review current medications for respiratory communicable diseases in the elderly, aiming to a better management of LRTIs in clinical practice.

Keywords: lower respiratory tract infections, elderly, controlled clinical trial, pharmacotherapy, antibiotics, drug resistance

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***Correspondence:**
Birong Dong
birongdong@163.com

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INTRODUCTION

The elderly may suffer from inappropriate medication due to decreased vision, memory loss, impaired cognition, low compliance, and unsupervised care. Hospitalization history, previous antibacterial therapy, physical decline, and mechanical ventilation are risk factors for LRTIs in this population. In the elderly, infections usually manifest as atypical symptoms such as lethargy, loss of appetite and mental disorders, thus inexperienced caregivers tend to ignore the above symptoms leading to the missed diagnosis and inappropriate use of antibiotics, increasing disability

and mortality in the elderly. One of the major causes of the growing LRTIs burden is increasing antimicrobial resistance. *Streptococcus pneumonia* (*S. pneumonia*), *Clamydia pneumonia*, *Staphylococcus aureus* and other bacterial pathogens remain the common causes of LRTIs. The resistances of these pathogens to macrolides and fluoroquinolones continue to increase at an alarming rate worldwide (Giske et al., 2008; Woodhead et al., 2011). For example, 48% of US isolates of *S. pneumoniae* tested were macrolide-resistant in 2014 (an increase from the 40% reported in 2008), and high-level macrolide resistance across the US was 33% (Jones et al., 2010). This is also the case in Europe (Ales et al., 2013). Aside from *S. pneumoniae*, atypical LRTIs-causing pathogens, such as *Mycoplasma pneumoniae*, have also produced increased antibiotic resistance (Asche et al., 2008). In the elderly, due to the long-term use of broad-spectrum antibiotics, immunosuppressants and invasive operations increase antibiotic resistance, ultimately leading to excess hospitalizations, treatment failures, and financial burdens. In addition, some physicians are not familiar with the physiological characteristics of the elderly or precautions for common medication, resulting in inappropriate use of antibiotics, such as: 1) Combination therapy with quinolones and warfarin increases the risk of bleeding in elderly patients, leading to QT prolongation; 2) Interaction between macrolides and statins may lead to rhabdomyolysis and acute kidney injury; 3) combination therapies with macrolides, fluoroquinolones, and sulfonylureas may cause severe hypoglycemia in the elderly. 4) Fluoroquinolones, macrolides, sulfonamides, nitrofurans, and β-lactams may cause damage to the central nervous system (CNS); and 5) fungal infections may be associated with the long-term use of antibiotics. In addition, antibiotics may affect the accuracy of diagnostic tests. Due to the high prevalence of LRTIs in the elderly both in hospital and outpatient setting (**Table 1**), the epidemiological differences, atypical clinical manifestations, and age-related variations in pharmacokinetics and pharmacodynamics make LRTIs management for the elderly more challenging, and standardized treatment at early stage of LRTIs is critical to reducing deaths and disability at present.

According to the 2017 Global Burden of Disease (GBD) Study (James et al., 2018), the burden of LRTIs in people older than 70 years old is still increasing in many regions (Troeger et al., 2017). Altered respiratory structure caused by aging (Song and Chang, 2017), impaired organ function (Poulose and Raju, 2014), changes of drug-susceptibility (Alldred et al., 2010), and chronic low grade inflammation (Boyd and Orihuela, 2011) together lead to the increased susceptibility to LRTIs. Meanwhile, the existing

TABLE 1 | Major pathogens and risk factors for pneumonia in community and LTCFs.

Pathogens	Prevalence of CAP in community elderly (%)	Prevalence of pneumonia in LTCFs elderly	Risk factors
S. pneumoniae (Mufson and Stanek, 1999; Waterer et al., 2001; Martinez et al., 2003; Baddour et al., 2004)	5–58	4–55	Used lactams, fluoroquinolones, macrolides in the past 3 months; COPD; History of pneumonia in past 12 months; Aspiration.
H. influenzae (Lau et al., 2006; Jean et al., 2009; Kuo et al., 2014)	2–29.4	0–22	Severe underlying disease; Used antibiotics in the past 3 months;
Staphylococcus aureus (Wunderink et al., 2003; Bernardo et al., 2004; Stevens et al., 2007; Kalil et al., 2013; Bradley, 2014)	0–7	0–33	Hospitalized in the past 3 months; Used antibiotics in the past 3 months; Living in LTCFs; Received intravenous therapy or dialysis for the past 30 days; Confirmed MRSA by etiological diagnosis; Comorbidity; Mental disorders.
Legionella (Miller, 1981; Edelstein et al., 1996; Genne et al., 1997; Vergis et al., 2000; Blazquez Garrido et al., 2005; Mykietiuk et al., 2005; Sabria et al., 2005; Haranaga et al., 2007; Varner et al., 2011)	0–17.5	0–6	Smoking; Chronic disease; Immunosuppression; Air conditioning and hot water system use.
Gram-negative enteric bacilli (Ortiz-Ruiz et al., 2004; Yakovlev et al., 2006; Peto et al., 2014)	0–12.4	0–14.3	Living in LTCFs; Tube feeding; Comorbidity; Cerebrovascular disease; Dementia; Use of Proton pump inhibitors (PPIs).
Pseudomonas aeruginosa (Ding et al., 2016; Francois et al., 2017; Bassetti et al., 2018; Ocheretyaner and Park, 2018; Riquelme et al., 2018)	1–17.1	0–6	Hospitalized in the past three months; Used antibiotics in the past 3 months; Aspiration; Impaired swallowing; Use of PPIs; Structural lung disease or severe bronchiectasis; Confirmed pseudomonas aeruginosa in the past 12 months; Severe illness (requires ventilator or admission of ICU).
Chlamydia pneumonia (Arnold et al., 2016; Marchello et al., 2016; Perrone and Quaglia, 2017; Webley and Hahn, 2017)	0–28	0–18	
Mycoplasma pneumoniae (Cao et al., 2017; de Groot et al., 2017; Sharma et al., 2017; Waites et al., 2017b)	1–13	1	

of comorbidities and aging, drug resistance, the prevalence and mortality of LRTIs in the elderly are much higher than other age groups, thus there is a huge demand for the development of novel pharmacotherapy for the elderly, and antibiotics seem to the cornerstone of LRTIs management (Katzan et al., 2003; Ma et al., 2013; Choi et al., 2018).

Based on the existing data of phase 3 clinical trials with the latest antibiotics, the purpose of this article is to recommend the most promising antibiotics for the treatment of LRTIs in the elderly. Meanwhile, we briefly reviewed current medications for respiratory communicable diseases in the elderly, aiming to obtain a better management of LRTIs in clinical practice.

METHODOLOGY

We comprehensively reviewed the research status of medication for LRTIs in the elderly and antibiotics, which are currently in advanced stages of development (phase 3 trial and beyond). After systematically retrieving the following sources including Pubmed, MEDLINE (OvidSP), and EMBASE (OvidSP) from October 2010 to July 2018, we have collected 87 clinical trials and manual screened out 58 trials (thirty-seven Phases 1 and 2 trials, eighteen Phase 3 trials, three Phase 4 trials, respectively), and finally elaborated the advantages and limitations of the application of novel antibiotics in clinical practice based on these trials.

All the random control trials (RCTs) included in our study share the following characteristics: trials included patients over 65 years of age who met at least three symptoms: cough, purulent sputum, dyspnea or pleurisy; if they had at least two abnormal vital signs, had at least one laboratory test result or clinical sign associated with LRTIs, and had radiologically confirmed pneumonia, these trials were classified as the risk classes in Pneumonia Severity Index (PSI), ranging from II to V. All of the trials we included were registered on ClinicalTrials.gov to assess the efficacy and safety of certain antibiotics. Population analysis, end points, and assessments were considered. Analysis populations including the intention-to-treat (ITT) population included all subjects who underwent randomization. The clinically evaluable (CE) population was defined as subjects who survive with resolution or improvement in symptoms and infections that further antibacterial therapy was not required. The microbiologic intention-to-treat (mITT) population was defined as all subjects in the ITT population who had a causative pathogen or pathogens identified at baseline by the culture of blood or respiratory specimens or using a culture-independent method. The clinical per-protocol population was defined as subjects in the ITT population who had a qualifying infection as defined by the trial entry criteria, had received a trial agent, had not received any antibacterial agent that was not as signed within the trial that could confound interpretation of the trial results, and had undergone an assessment of results during the protocol defined window. The microbiologic per-protocol population included the patients in both the clinical per-protocol population and the mITT population. Regarding end points, firstly the primary efficacy end point was evaluated as early clinical response (ECR), which was defined as survival with improvement of one or more levels relative to baseline in two or more symptoms of pneumonia and no worsening of one or more levels in other symptoms of pneumonia, without receipt of rescue antibacterial therapy. Generally, ECR was assessed 24–72 h after the first dose of trial drug in the ITT population. The secondary end point was investigator-assessed clinical response at a post-treatment evaluation 5 to 10 days after the last dose, with clinical response defined as resolution or improvement in signs or symptoms to the extent that further antibacterial therapy was unnecessary. At the same time, we also evaluated the adverse reactions of antibiotics, including mild adverse events, serious adverse events (SAEs) defined as adverse events emerged after treatment initiation, and treatment discontinuation. The mortality in both arms was also analyzed for the safety of certain agents.

ANTIBIOTICS FOR COMMUNITY ACQUIRED PNEUMONIA

Fluoroquinolone

In recent years, new fluoroquinolone agents (**Table 2**), such as delafloxacin, nemonoxacin and zabofloxacin, have been identified as effective against existing fluoroquinolone-resistant pathogens. These new fluoroquinolone agents target both topoisomerase IV and DNA gyrase with stronger affinities, resulting in inhibition of bacterial DNA replication (Kollef and Betthauser, 2019), reducing mutant selection and toxic side effects, and resulting superior potent activity against the most common community acquired pneumonia (CAP) pathogens (Pfaller et al., 2017c). Delafloxacin is effective against Gram-positive bacteria, including *methicillin sensitive Staphylococcus aureus* (*MSSA*), *methicillin resistant Staphylococcus aureus* (*MRSA*), *Moraxella catarrhalis* (*M. catarrhalis*), and *S. pneumoniae*. While nemonoxacin is effective against Gram-positive bacteria, including multidrug-resistant *S. pneumoniae*, *MRSA*, ertapenem-nonsusceptible *Enterobacteriaceae*, *Legionella*, *Chlamydophila*, and *Mycoplasma*. Antibacterial activity of zabofloxacin against MSSA and MRSA is similar to gemifloxacin, but 2–16 times stronger than that of moxifloxacin and ciprofloxacin (Park et al., 2006).

Nemonoxacin

RCT (NCT01529476) of a phase 3 was conducted in CAP patients receiving nemonoxacin 500 mg or levofloxacin 500 mg orally once daily for 7–10 days. A total of 527 patients (18–70 years old) were randomized to treat with nemonoxacin or levofloxacin. The clinical cure rates at test of cure (TOC) visit were 94.3% for nemonoxacin and 93.5% for levofloxacin in the mITT population. The microbiological success rates were 92.1% for nemonoxacin and 91.7% for levofloxacin in the mITT population. Nemonoxacin was as effective and safe as levofloxacin in the treatment of adult CAP patients in terms of clinical cure rates, microbiological success rates, and safety profile (Yuan et al., 2019). For other phrase 3, non-inferiority trials (NCT02205112, NCT03551210), in which old patients accounted for the majority of the participants, had repeatedly confirmed the safety and

TABLE 2 | Summary of advantages and limitations of the novel antibiotics.

Antibiotics	Mechanism of action	Frequency of interactions	Side effects	Frequency of dosing	Phase of study	FDA/ EMA approved	Intravenously or orally	Recommend	MIC_{90} of novel antibiotics compared with existing antibiotics
Nemonoxacin	–Target both topoisomerase IV and DNA gyrase. –New fluoroquinolone.	LOW	TRANSIENT ELEVATION OF AMINOTRANSFERASE.	ONCE DAILY	3	YES	ORAL AND IV	A first-line medication.	–CS-MRSA: The MIC_{90} (μg/mL) of nemonoxacin, levofloxacin, moxifloxacin are 0.25, 0.25, 0.5, respectively. –CR-*MRSA*: The MIC_{90} (μg/mL) of nemonoxacin, levofloxacin, moxifloxacin are 0.5, 32, 8, respectively (Barriere, 2014).
Zabofloxacin	–Target both topoisomerase IV and DNA gyrase. –4th generation quinolone (fluoroquinolone).	NOT PROVIDED	–Mild, self-limiting. –gastrointestinal (GI) symptoms and allergic reactions.	Once daily	3	NO	ORAL ONLY	Not recommend.	–*PSSP* & *PISP* & *PRSP*: The MIC_{90} (mg/mL) of zabofloxacin, ciprofloxacin sparfloxacin are 0.03, 2, 0.5, respectively (Barriere, 2014). –*MRSA*: The MIC_{90} (μg/mL) of zabofloxacin, moxifloxacin, levofloxacin are 2, 8, and 16, respectively (Mohamed et al., 2019).
Delafloxacin	–Target both topoisomerase IV and DNA gyrase. –4th generation quinolone (fluoroquinolone).	Low	–Favorable AEs profile. –nausea, diarrhea.	Q12h	III (STILL PENDING)	NO	Oral and IV.	Not recommend	–MRSA: The MIC_{90} (mg/L) of delafloxacin, moxifloxacin are 0.004, 0.032 respectively. –MSSA: The MIC_{90} (mg/L) of delafloxacin, moxifloxacin are 0.004, 0.125 respectively (Siala et al., 2016).
Omadacycline	–A unique alkylaminomethyl side chain at the c9 position of the tetracycline	Low	–Mild gastrointestinal symptoms. –CHANGES OF HR AND QT INTERVAL.	Once daily	3	Yes	Oral and IV	–Moderate. –For the elderly without cardiac electrophysiological abnormalities.	–*Chlamydia pneumoniae*: The MIC_{90} (mg/mL) of omadacycline, levofloxacin, moxifloxacin are 0.25, 0.5, 1, respectively (Roblin et al., 1997). –*Mycoplasma pneumoniae* : The MIC_{90} (μg/mL) of omadacycline, doxycycline, tetracycline are 0.25, 0.5, 0.5, respectively (Waites et al., 2016).
Solithromycin	–The first fluoroketolide, whichbinds to an additional site on rRNA.	HIGH .	SEVER HEPATIC TOXICITY	Once daily	3	NO	Oral and IV	Not recommend	–*MRSA*: The MIC_{90} (mg/L) of solithromycin, telithromycin, azithromycin, clarithromycin are 2, 4, 2, 8, respectively. –*Legionella pneumoniae*: The MIC_{90} (μg/mL) of solithromycin, azithromycin are 0.03, 1, respectively (Waites et al., 2016).
Ceftaroline	A strong affinity for PBPs – Destroy cell wall formation.	Low	Mild and self-limiting	THRICE DAILY	3	Yes	IV ONLY	–Moderate. –For elderly Clearance ≥30 ml/min. –For elderly without QT prolongation.	–*Ceftriaxone-nonsusceptible (NS) s. pneumoniae*: The MIC_{90} (μg/mL) of ceftaroline, ceftriaxone are 0.12, ≥2, respectively. –*Amoxicillin-clavulanate-NS s. pneumoniae*; The MIC_{90} (μg/mL) of ceftaroline, amoxicillin-clavulanate are 0.12, ≥4, respectively. –Levofloxacin-NS s. pneumoniae; The MIC_{90} (μg/mL) of ceftaroline, Amoxicillin-clavulanate are 0.12, ≥1, respectively (Pfaller et al., 2017b).
Ceftobiprole	A strong affinity for the PBPs	Low	Mild and self-limiting	THRICE DAILY	3	NO	IV ONLY	–Not recommend. –Data in some centers were unreliable.	–*Amoxicillin-resistant S. pneumoniae*: The MIC_{90} (μg/mL) of ceftriaxone, ceftaroline, ceftobiprole are 0.25, 0.06, 0.06, respectively (Green et al., 2014).

(*Continued*)

TABLE 2 | Continued

Antibiotics	Mechanism of action	Frequency of interactions	Side effects	Frequency of dosing	Phase of study	FDA/ EMA approved	Intravenously or orally	Recommend	MIC_{90} of novel antibiotics compared with existing antibiotics
Lefamulin	Inhibit protein synthesis by binding to the bacterial ribosome.	–HIGH, –Interact with azole antifungals and midazolam.	Mild	TWICE DAILY	3	NO	Oral and IV	–Moderate. –For elderly without taking azole antifungals, midazolam.	–*Mycoplasma* pneumoniae, macrolide-susceptible: The MIC_{90} (μg/mL) of lefamulin, solithromycin, moxifloxacin, Tetracycline, Doxycycline are 0.02, 0.5, 0.25, 1, 0.25, respectively. –*Mycoplas*ma. pneumoniae, macrolide-resistant: The MIC_{90} (μg/mL) of lefamulin, solithromycin, moxifloxacin, tetracycline, doxycycline are 0.02, NA, 0.25, 1, NA, respectively (Waites et al., 2017a).
Pristinamycin	Inhibits protein synthesis by binding to the bacterial ribosome 50s subunit	HIGH	Mild	THRICE DAILY	3	NO	ORAL ONLY	Not recommend	–*MRSA*: The MIC_{90} (μg/mL) of pristinamycin, linezolid, vancomycin, teicoplanin are 0.5, 0.5, 2, 2, respectively (Zmira et al., 2005).
Iclaprim	Selectively and potently inhibits dihydrofolate reductase.	Low	Mild	Twice daily	II	NO	IV ONLY	Not recommend (HAP)	Vancomycin-*NS MRSA*: The MIC_{90} (μg/mL) of iclaprim, vancomycin are 0.25, > 4, respectively (Huang et al., 2017).
Telavancin	–Interfering transpeptidation, polymerization. –Increases potassium and ATP leakage	–HIGH. –Interact with digoxin, warfarin, benzodiazepines.	Mild	Once daily	4	NO	IV ONLY	Moderate (HAP)	–*MRSA*: The MIC_{90} (μg/mL) of telavancin, vancomycin, linezolid, levofloxacin are 0.06, 1, 1, > 4, respectively. –*S. pneumoniae*: The MIC_{90} (μg/mL) of telavancin, vancomycin, linezolid, levofloxacin are ≤0.015, 0.5, 1, 1, respectively (Duncan et al., 2017).
Tedizolid	Additional interactions with conserved regions of the ribosomal subunit and the d-ring substituent.	Low	REMAINS TO BE SEEN.	Once daily	3 (UNFINISHED)	NO	IV and oral	Not recommend (HAP)	–*MRSA*: The MIC_{90} (μg/mL) of tedizolid, linezolid, vancomycin, Levofloxacin are 0.12, 1, 1, > > 4, respectively (Duncan et al., 2017).
Levofloxacin	Target both topoisomerase iv and dna gyrase	Interact with Warfarin, theophylline, NSAIDs	Phototoxicity, systemic active allergic reactions, hepatotoxicity, severe CNS toxicity	Twice daily	IV	YES	IV and oral	–Moderate. –Good post-marketing response.	See above
Ceftriaxone	A higher affinity for PBPs. –Destroy cell wall formation.	Low	Eosinophilia, leukopenia, thrombocytopenia.	Once daily	IV	YES	IV only	–Moderate. –Good post-marketing response.	See above
Moxifloxacin	Topoisomerase ii, iv inhibitor	Low	Diarrhea, fever, CNS, toxicity	Once daily	IV	YES	IV and oral	–Moderate. –Good post-marketing response.	See above
Amoxicillin	A higher affinity for pbp and can destroy cell wall formation more quickly and effectively	Low	Mild and self-limiting,diarrhea, headache, nausea, anaphylaxis	Thrice daily	IV	YES	IV and oral	–Moderate. –Good post-marketing response.	See above
Linezolid	Interactions with conserved regions of the 23s ribosomal subunit and the d-ring substituent of tedizolid.	Low	Mild and self-limiting,diarrhea, headache, nausea	Thrice daily	IV	YES	IV and oral	–Moderate(HAP) –Good post-marketing response.	See above
Vancomycin	Inhibit the synthesis of bacterial RNA and cell walls, and change the permeability of cell membranes.	Low	–Acute kidney injury–Vestibulocochlear nerve damages	Twice or quartic daily	IV	YES	IV only	–Moderate(HAP) –Good post-marketing response.	See above

Reasons for recommending or not recommending have been marked in capital letters, such as IV, II, YES, NO, SEVER HEPATIC TOXICITY; MIC_{90}, the minimal inhibitory concentration required to inhibit the growth of 90% of isolates; CR-MRSA, ciprofloxacin-resistant and methicillin-resistant Staphylococcus aureus; PSSP, penicillin-susceptible Streptococcus pneumoniae; PISP, penicillin-intermediate S. pneumoniae; PRSP, penicillin-resistant S. pneumoniae; MRSA, methicillin resistant Staphylococcus aureus.

efficacy of nemonoxacin in the treatment of CAP (van Rensburg et al., 2010; Chang et al., 2019).

Values to the Elderly

1) Novel mechanism of action: nemonoxacin targets both topoisomerase IV and DNA gyrase, inhibiting DNA synthesis required to bacterial growth (Li et al., 2015); 2) Frequency of interactions: when the creatinine clearance is <50 mL/min, the dosage of levofloxacin need to be adjusted, while nemonoxacin does not induce or inhibit CYP1A2, 2B6, 2C8, 2C9, C19, and 3A4 isozymes (Cao et al., 2014). No dosage adjustment is required for the elderly with impaired renal or hepatic function. 3) Side effects: unlike other commercially available fluoroquinolone agents (moxifloxacin, levofloxacin), nemonoxacin does not exhibit evidences of phototoxicity, systemic active allergic reactions, significant hepatotoxicity, or severe CNS toxicity (Liang et al., 2013; Zhang et al., 2016). 4) Dosing regimen: In a systemic review and meta-analysis of RCTs demonstrated that compared with 500 mg levofloxacin, nemonoxacin (500 mg or 750 mg) was more safe in cardiac conduction as measured by ECG QTc prolongation (Chang et al., 2019). In addition, a single-dose escalation (nemonoxacin 25–1,250 mg) study shows that there were no clinically significant changes in corrected QT in healthy Chinese volunteers (Luke et al., 2010), but the 750 mg dosage had a significantly higher risk of adverse effects than the 500 mg dosage, so the nemonoxacin 500 mg regimen may be adequate for the treatment of CAP (Roychoudhury et al., 2016). The oral dosage of nemonoxacin is 500 mg once daily while it is 100 mg twice daily for levofloxacin, making nemonoxacin a potential therapy for the elderly with LRTIs.

Zabofloxacin

Multicenter, non-inferior RCT (NCT01658020) of a phase 3 evaluated the safety and efficacy of oral zabofloxacin (367 mg once daily for 5 days) vs oral moxifloxacin (400 mg once daily for 7 days) in treating acute bacterial exacerbation of chronic obstructive pulmonary disease (COPD); 345 participants with moderate acute bacterial exacerbation COPD were selected. In a subgroup of patients without chronic bronchitis but suffering from LRTIs, antibacterial efficacy of zabofloxacin and moxifloxacin therapies were observed, and the cure rates were 85.9% and 84.2%, respectively. No statistical differences of acute AEs and serious AEs were detected between the two arms (Rhee et al., 2015).

Values to the Elderly

1) Novel mechanism of action: zabofloxacin can inhibit DNA gyrase and topoisomerase IV, thus inhibiting the bacterial DNA replication (Park et al., 2010). Zabofloxacin shows potent *in vitro* activity against *S. pneumoniae* isolates that caused invasive pneumococcal disease, even levofloxacin-resistant strains (Kwon et al., 2006). 2) Side effects: adverse effects include nausea, hypotension, somnolence, and an increase of blood phosphokinase, which are common and minor and will subside spontaneously. Meanwhile, no QT prolongation was detected (Kocsis et al., 2016). 3) Dosing regimen: dosing regimen is relatively simple, requiring only one dose per day.

Delafloxacin

RCT (NCT02679573) of a phase 3 on comparison of delafloxacin and moxifloxacin for the treatment of adults with CAP was completed. At present, the results of this trial are still pending. Based on this situation, we do not recommend delafloxacin as a first-line agent for LRTIs in the elderly.

Tetracycline

Omadacycline

Omadacycline (**Table 2**) was a novel once-daily aminomethylcycline antibiotic, and became the second tetracycline antibiotic approved by the FDA in 2018. Omadacycline has antimicrobial activity against Gram-positive, Gram-negative, anaerobic, and atypical pathogens (Dougherty et al., 2019). Omadacycline has a higher coverage against MRSA, penicillin- and multidrug-resistant *S. pneumoniae*, and Vancomycin-resistant enterococci (VRE). Omadacycline also has good activity against *H. influenza*, *M. catarrhalis*, *M. pneumoniae*, *L. pneumophila*, *Enterobacteriaceae*, *Ureaplasma* spp., *Bacillus anthracis*, *Yersinia pestis*, and *Clostridium difficile* (Pfaller et al., 2017a).

A phase 3 trial (NCT02531438) on the efficacy and safety of omacycline for CAP patients had been successfully completed. A total of 772 CAP patients (PSI: II–IV) were randomly enrolled into two groups of the equal size. Patients in the two groups took intravenous omadacycline or moxifloxacin in the first three days, and then transitioned to oral omadacycline or moxifloxacin, respectively. Overall, 41.9% of patients in the ITT populations were older than 65 years old, and 85.4% had PSI risk class of III or IV in this population. Study showed no significant differences between the two arms in terms of ECR, 5–10 days of clinical responses, and incidences of AEs. All the patients who died were older than 65 years old (eight in the omadacycline group and four in the moxifloxacin group). These deaths might be caused by progression of the underlying pneumonia or respiratory compromise, HAP, cardiac or vascular events, and cancer. Neither group had clinically relevant changes from baseline in vital signs, laboratory tests, nor ECG findings. Researchers concluded that deaths in both groups were related to underlying disease rather than these two antibiotics. In summary, the efficacy of omadacycline in the treatment of CAP was not inferior to that of moxifloxacin (Stets et al., 2019).

Values to the Elderly

1) Novel mechanism of action: the chemical structure of omadacycline contains a unique alkylaminomethyl side chain at the C9 position of the tetracycline. 2) Frequency of interactions: omadacycline has mild drug interactions and favorable safety profiles. *In vitro*, researchers found that omadacycline does not affect cytochrome P450, and that the most common AEs of omadacycline are gastrointestinal symptoms (2019). No clinically significant differences in omadacycline pharmacokinetics were

observed based on age. There is no need for the elderly with impaired or and hepatic function to adjust dose of omadacycline. 3) Dosing regimen: dosing regimen is relatively simplistic as only one dose is needed per day. This regimen greatly reduces the likelihood that an impaired-cognitive patient take repeated medicine or forget to take the medicine.

Macrolide

Solithromycin

Solithromycin (**Table 2**) is a novel 4th generation macrolide. It's the first fluoroketolide to complete phase 3 clinical trials and show activity against the pathogens associated with LRTIs, including macrolide/penicillin-resistant isolates of *S. pneumoniae*. Solithromycin influence the formation and function of 50S ribosomal subunit, causing the frame-shift mutation during translation (Still et al., 2011). Due to the lack of a cladinose moiety, it does not induce erm(B)-mediated resistance (3Rd et al., 2015). And it is less susceptible to mef(A)-mediated efflux than other macrolides as a result of its increased ribosomal binding and greater intrinsic activity (Darpo et al., 2017a).

One trial (NCT01756339) compared the antibacterial efficacy and safety of oral solithromycin for the treatment of CAP in a 114 central non-inferiority RCTs. During this study, patients were randomly assigned (1:1) to receive either oral solithromycin or moxifloxacin. The results showed that 78.2% participants had an ECR in the solithromycin group compared with 77.9% in the moxifloxacin group, showing equivalent efficacy of solithromycin for the primary endpoint. Subjects over 65 years of age with a history of asthma and COPD had higher success rates for ECR and short term follow-up than those without COPD. In addition, the ECR rate is higher in the 75-year-old group, which may be related to the immunomodulatory effects of solithromycin among all groups (Barrera et al., 2016). In another phase 3 trial (NCT01968733), the efficacy and safety of intravenous-to-oral solithromycin were assessed against intravenous-to-oral moxifloxacin for the treatment of CAP. In this trial, the ECR in the ITT population aged 65–74 years old and older than 75 year old showed non-inferiority of solithromycin for the primary endpoint, respectively. The incidence rate of serious AEs was comparable between groups with no significance (File et al., 2016).

Values to the Elderly

Solithromycin has many advantages to be provided for the elderly population. 1) Novel mechanism of action: solithromycin demonstrates increased ribosomal binding in comparison with other macrolides. Meanwhile, as the first fluoroketolide, fluorine contributes to tighter binding and increased activity, and the potential for resistance appears to be low (Darpo et al., 2017b). 2) Frequency of interactions: Due to it is inhibition of the CYP3A isoenzyme pathway it has frequent drug-drug interactions like other macrolides such as erythromycin and clarithromycin. 3) Side effects are mild and relatively low in frequency, however there are concerns of severe hepatic toxicity that require further evaluation (Hook et al., 2015). 4) Dosing regimen: solithromycin also has a simple dosing regimen, with once-daily dosing for the treatment of CAP. For the elderly with poor vision, memory loss, cognitive impairment, and low self-adherence, it's the preferred choice. Moreover, solithromycin is available in both oral and intravenous (IV) formulation, and is highly potent with effective bacteriostatic properties and eradication rates from a pharmacodynamics (PD) perspective.

Cephalosporin

Cephalosporins, including ceftobiprole and ceftaroline, is the "new-generation" which is effective against MRSA, MSSA, penicillin-resistant *S. pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Green et al., 2014).

Ceftaroline

In 2010, ceftaroline (**Table 2**) was approved by the FDA and European Medicines Agency (EMA) for the treatment of CAP. Its broad-spectrum activity, especially its potent antibacterial activity against Gram-positive bacteria, makes ceftaroline an ideal antibiotic for the treatment of CAP. The efficacy and safety of ceftaroline are established in two milestone studies FOCUS 1 and FOCUS 2. FOCUS 1(NCT00621504) enrolled 613 CAP patients 49.2% of whom were aged ≥65 years old. The experimental group was treated with intravenous ceftaroline 600 mg Q12 h × 5–7 days, and the control group was treated with ceftriaxone and clarithromycin. FOCUS 2 (NCT00509106) recruited 627 CAP patients with the same criteria. Almost half (46.8%) of the patients across both groups were aged ≥65 years old. Both arms took the same intervention as FOCUS 1, and only clarithromycin was not used as adjuvant therapy in FOCUS 2. In both FOCUS 1 and 2, ceftaroline and ceftriaxone were well tolerated, with similar rates of AEs, serious AEs, deaths and discontinuations (File et al., 2011; Low et al., 2011). Another published RCT (NCT01371838) included 771 Asian CAP (PORT risk class III–IV) patients meeting the same criteria as FOCUS. The experimental group used exactly the same intervention as in FOCUS, and the control group used double dosage of ceftriaxone. The results show that ceftaroline is superior to ceftriaxone in clinically evaluable (CE) and mITT population. There was no significant difference in safety between the two agents (Zhong et al., 2015). A Phase 4 multicenter study (NCT01666743) was proposed to specifically evaluate the safety and efficacy of ceftaroline in the treatment of CAP in patients 65 years of age, but the study was withdrawn for unknown reasons. Other studies on the safety and efficacy of ceftaroline for CAP are being recruited (NCT02735707) or have not yielded results (NCT03025841).

Values to Elderly

(1) Novel mechanism of action: compared with other penicillin or cephalosporin β-lactam antibiotics, ceftaroline has a higher affinity for penicillin-binding proteins (PBPs) and can destroy cell wall formation more quickly and effectively (Justo et al., 2015). Its broad-spectrum activity, especially its potent antibacterial activity against resistant Gram-positive bacteria, makes it an ideal drug for the treatment of CAP. (2) Frequency of interactions and side effects: side effects of solithromycin are mild, and the frequency was relatively low. For elderly patients

with moderate impaired renal function, ceftaroline does not require dose adjustment. (3) Dosing regimen: regimen is simple, and intravenous infusion twice a day is sufficient.

Ceftobiprole

Ceftobiprole (**Table 2**) has good activity against Gram-positive pathogens. It has species-dependent activity against Gram-negative pathogens (Curcio, 2014).

Two large scale *in vitro* studies (Farrell et al., 2014; Hodille et al., 2017) of ceftobiprole showed that ceftobiprole had strong activity against *MSSA* (100%, 100% susceptible, respectively), MRSA (98.3%, 99.3% susceptible, respectively), *S. pneumoniae* (99.3%, 99.7% susceptible, respectively), and the majority of *Enterobacteriaceae* (87.3%, 82.5% susceptible, respectively). The potency of ceftobiprole against *P. aeruginosa* (64.6%, 72.7% susceptible, respectively) was similar to that of ceftazidime (Kresken et al., 2011). For elderly people in long-term care facilities (LTCFs), agents are necessary for the coverage of rare pathogens, while ceftobiprole has good antibacterial activity against common pathogens of LTCFs, such as *Enterobacteriaceae* and *P. aeruginosa*. Nowadays, ceftobiprole is approved in several European countries for the treatment of CAP and HAP (excluding VAP) (Scheeren, 2015).

The safety and efficacy of ceftobiprole have been demonstrated in two phase 3 trials on patients with CAP and HAP (excluding VAP). The first study (NCT00326287) demonstrated that intravenous ceftobiprole had equivalent efficacy to ceftriaxone with or without linezolid. Details: clinical cure rates for CAP patients were 86.6% vs 87.4% (clinical evaluate population, 95%CI, −6.9, 5.3), and 76.4% vs 79.3% (ITT population, 95% CI, −9.3, 3.6). Pneumonia-specific mortality within the first 30 days was very low in both groups. In addition, common and serious AEs in the ceftobiprole arm were mild and comparable to those in the ceftriaxone arm (Nicholson et al., 2012). The second RCT (NCT00210964) demonstrated ceftobiprole was non-inferior to ceftazidime with or without linezolid. It is worth noting that cure rates for VAP patients were 23.1% vs 36.8% and 37.7% vs 55.9%, suggesting that ceftobiprole was unsuitable for the treatment of VAP (Awad et al., 2014). A retrospective study of the above RCTs evaluated the early clinical improvement in subgroups of high-risk patients. In some subgroups of high-risk patients with CAP (such as patients over 75 years old, or or CAP patients with COPD, or HAP patients with more than 10 baseline comorbidities), particular and significant results were observed that seemed to favor the ceftobiprole over comparators (Pooley et al., 2014).

Values to Elderly

(1) Novel mechanism of action: ceftobiprole with a strong affinity for the PBPs, is responsible for the antibacterial activity of staphylococci and pneumococci (Falco et al., 2018). For pneumonia patients with comorbidities, ceftobiprole with the strong bactericidal effect can quickly improve clinical symptoms and ensure a better prognosis. (2) Frequency of interactions: ceftobiprole elimination is not expected to be significantly affected, as this is a minor elimination route, but dose adjustment is necessary for subjects with the renal impairment (Pfaller et al., 2019). (3) Side effects: for comorbid patients older than 75 years old, the incidence of adverse events caused by ceftobiprole is similar to that of non-high-risk patients, suggesting that ceftobiprole is safe and effective for high-risk groups. In addition, ceftobiprole is less likely to cause an antibiotic-related intestinal flora disorder (Horn et al., 2017).

Pleuromutilin

Lefamulin

Lefamulin (**Table 2**) is a potent semi-synthetic antibacterial agent belonging to a novel class known as the pleuromutilins. Lefamulin's *in vitro* antibacterial profile includes the most important bacterial pathogens causing LRTIs. The antibacterial spectrum comprises *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, the atypical respiratory pathogens, *MRSA*, β-haemolytic *streptococci*, and *Enterococcus faecium* (Waites et al., 2017a; Veve and Wagner, 2018). Moreover, as demonstrated in cross-resistance studies, lefamulin remains active against clinical isolates resistant to the following antibiotics: macrolides, lincosamides, streptogramin B, oxazolidinones, tetracyclines, β-lactams, quinolones, trimethoprim-sulfametoxazole, mupirocin, and vancomycin (Mendes et al., 2019; Paukner et al., 2019).

The phase 3 clinical trial, LEAP1 (NCT02559310), for evaluating the safety and efficacy of lefamulin for the treatment of CAP has been completed. Participants with CAP were randomized 1:1 to receive lefamulin at 150 mg IV every 12 h or moxifloxacin at 400 mg once daily. After six doses, patients could be switched to an oral administration if pre-specified improvement criteria were met. If *MRSA* was suspected, linezolid was added to moxifloxacin. In LEAP1, patients aged over 65 years old accounted for 47.8% and 39.3% of the lefamulin and moxifloxacin groups, respectively. At this age, lefamulin was non-inferior to moxifloxacin for ECR, or investigators assessed clinical response (IACR). Lefamulin has a low incidence of drug resistance and minimal cross-resistance with other types of antibiotics, making it a new monotherapy for elderly CAP (File et al., 2019). The oral dosage form of lefamulin is under the investigation in LEAP 2 (NCT02813694), and the primary endpoint is similar to LEAP 1. A major difference in study design includes the use of only oral drugs without the addition of linezolid in the moxifloxacin group. The LEAP 2 results are expected to be available in the second half of 2019.

Values to Elderly

In LEAP 1, patients ≥65 years of age accounted for 47.8% and 39.3% of the lefamulin and moxifloxacin groups, respectively. At this age, lefamulin was non-inferior to moxifloxacin for ECR or IACR. (1) Novel mechanism of action: inhibit protein synthesis by binding to the bacterial ribosome 50S subunit (Veve and Wagner, 2018), which ensures that lefamulin has a low incidence of drug resistance and minimal cross-resistance with other types of antibiotics, making it a new monotherapy for elderly CAP. (2) Frequency of interactions: lefamulin has little inhibitory effect on CYP3A, however, it's worth noting that its high protein binding capacity could lead to drugs interaction (Waites et al., 2017a). (3) Side effects: lefamulin only

has mild side effects and is highly effective against common CAP pathogens (Mendes et al., 2019).

Streptogramins

Pristinamycin

Pristinamycin (**Table 2**) is a streptococcal-type antibiotic produced by *Streptomyces faecalis*. It inhibits protein synthesis by binding to the bacterial ribosome 50S subunit (Nespoulous et al., 2018). Pristinamycin has strong antibacterial activity against MRSA, MSSA, *H. influenzae*, and *S. pneumonia* (Cooper et al., 2014). In addition, pristinamycin has a synergistic antibacterial effect with vancomycin (Reid et al., 2010).

A phase 4 study (NCT02332577) intended to evaluate the safety and efficacy of pristinamycin in the treatment of mild CAP is expected to be completed in May 2021.

Values to Elderly

It is noteworthy that the above trials excluded patients with moderate and severe CAP, which may limit its generalizability. In addition, pristinamycin has only oral formulation, so it's unlikely that it will ever have a role in treating old patients with severe CAP. We do not recommend pristinamycin as a promising treatment for CAP.

ANTIBIOTICS FOR HOSPITAL ACQUIRED PNEUMONIA OR LTCFS ACQUIRED PNEUMONIA

Dihydrofolate-Reductase Inhibitor

Iclaprim

Iclaprim (**Table 2**) is a broad-spectrum diaminopyrimidine antibiotic that inhibits the dihydrofolate reductase and does not cross react with human enzyme (Laue et al., 2007). Iclaprim is being developed to treat serious respiratory infections, such as hospital acquired pneumonia (HAP), attributed to multidrug-resistant Gram-positive pathogens and cystic fibrosis caused by *S. aureus* (Huang et al., 2018; Huang et al., 2019). Until now, only one phase 2 clinical trial (NCT00543608) has focused on exploring iclaprim's efficacy on HAP caused by Gram-positive bacteria, but the trial has not been completed. Therefore, iclaprim is not recommended as a routine treatment for elderly HAP.

Lipoglycopeptides

Telavancin

Telavancin (**Table 2**) is a novel semi-synthetic lipoglycopeptides that is active against multidrug resistant (MDR) staphylococci, enterococci, and streptococci. Telavancin was approved by the FDA in 2013 for the HAP and ventilator-associated bacterial pneumonia (VABP). Telavancin has high antibacterial efficacy against *S. aureus* (MIC_{90} = 0.5 mg/L), *S. epidermidis* (MIC_{90} = 0.5 mg/L) (both *MSSA* and *MRSA*), *VISA* (MIC_{90} = 0.5 mg/L), *Streptococcus* (MIC_{90} = 0.03mg/L), and *VanB protein enterococcus*(MIC_{90} = 2 mg/L), but has a poor effect on *VRSA* (MIC_{90} = 8 mg/L) and VanA protein, enterococcus (MIC_{90} = 8 mg/L) (Hassoun et al., 2017). Two RCTs named "ATTAIN" enrolled in more than 700 HAP patients who were randomized to receive telavancin (10 mg/kg, QD) or vancomycin (1 g, Q12H). The results of the study indicate that telavancin was no worse than vancomycin in terms of the clinical cure rate of TOC visits in both ATTAIN studies. The subgroup analysis also showed that telavancin had a better effect on simple *S. aureus* infection, while vancomycin had a better effect on mixed infection of Gram-positive and Gram-negative bacteria. For MRSA, telavancin and vancomycin have similar effects and similar rates of AEs, but telavancin causes a higher proportion of people with elevated serum creatinine levels than vancomycin (10% vs 8%) (Barriere, 2014). In summary, ECG monitoring is necessary for elderly patients with a history of QT prolongation. At the same time, patients using telavancin should be monitored for coagulation parameters before and after dosing (Al Jalali and Zeitlinger, 2018).

Values to Elderly

1) Novel mechanism of action: telavancin has a dual antibacterial mechanism of action, which is to inhibit bacterial cell wall synthesis by interfering with cross-linking (transpeptidation) and polymerization (Rubinstein et al., 2011). Meanwhile, telavancin can cause cell death by increasing membrane permeability, resulting in the leakage of intracellular potassium and ATP. 2) Frequency of interactions: telavancin has mild inhibitory effect on CYP3A (Das et al., 2017), thus it can also be used in elderly with hepatic dysfunction. However, it's worth noting that its high protein binding capacity could lead to drug to drug interaction (Al Jalali and Zeitlinger, 2018). 3) Side effects: telavancin only has mild side effects and high potency for common CAP pathogens. 4) Dosing regimen: the single-dosage or two-dosage regimen can greatly improve the compliance of old patients. In addition, compared with vancomycin, telavancin has the advantages of potent antibacterial activity against *MRSA*, *VISA* and even *VRSA* as well as long half-life. It has good antibacterial activity. It can fill in gaps when vancomycin is resistant (Barriere, 2014). Based on all the above details, we moderately recommend telavancin as a promising antibiotic for LRTIs in elderly.

Oxazolidinone

Tedizolid

Tedizolid (**Table 2**) was approved by the FDA for the treatment of acute bacterial skin and skin structure infections (ABSSSIs) in 2016. Tedizolid is one of very few prospective agents with a spectrum of activity against MRSA and VRE, which are common pathogens in nosocomial pneumonia (Flanagan et al., 2013). Tedizolid shares many structural features with linezolid and has increased antimicrobial potency than linezolid. Many studies have confirmed that the antibacterial potential of tedizolid for linezolid-susceptible and linezolid-resistant Gram-positive pathogens is much higher than that of linezolid (Brown and Traczewski, 2010). To date, no phase 3 trials assessing efficacy of tedizolid for the treatment of HAP have been completed. Until now, no documented short-term animal and clinical studies have

reported neuropathies or thrombocytopenia associated with tedizolid, but the safety of tedizolid for long-term administration remains to be seen.

A randomized phase 3 study (NCT02019420) of the safety and efficacy of tedizolid in comparison with linezolid in patients with HAP and VAP is currently ongoing. The primary endpoint is to determine the non-inferiority (NI) in all-cause mortality (ACM) within 28 days after the randomization of intravenous tedizolid phosphate compared with intravenous linezolid in the ITT Analysis Set in ventilated participants with Gram-positive nosocomial pneumonia. The result is expected to be completed by February 2018, but the researchers have not announced the results of the trial.

Values to Elderly

1) Novel mechanism of action: additional interaction with conserved regions of the ribosomal subunit and the D-ring substituent of tedizolid contributes to its strong antibacterial potential. The level of tedizolid penetration into epithelial lining fluid (ELF) and alveolar macrophages (AM) is much higher than free-drug exposures in plasma (Housman et al., 2012). 2) Side effects: in the presence of linezolid resistance or hematologic side effects (Lodise et al., 2016), tedizolid is a better choice. 3) Frequency of interactions: for the elderly with any degree of hepatic and renal dysfunction, no dose adjustment was warranted in elderly to achieve therapeutic goals. 4) Dosing regimen: in addition, its better bioavailability, food-independent efficacy, and simple dosing regimens that support once daily administration, making tedizolid popular with clinicians.

SPECIAL CONSIDERATION

We briefly review the current status of pharmacotherapies for special types of LRTIs in elderly. We searched the following sources including Pubmed, MEDLINE (OvidSP), EMBASE (OvidSP), from July 2015 to July 2018. We finally concluded that the risk of LRTIs is much higher in immunocompromised old adults with diabetes than healthy elderly. Pharmacotherapies for old patients with special types of LRTIs (fungal pneumonia, respiratory HCoVs, influenza) are basically the same as for all age groups, but at the same time, considering the health status (frailty, long-term lying in bed, recurring infection and excess hospitalization, cognitive impairment), comorbidities, medication and vaccination history are also important for developing individualized medication regimens.

Diabetes Mellitus

A retrospective study of patients with diabetes reveals a high correlation between prevalence of infection and fasting blood glucose (FBG) in the elderly (Rayfield et al., 1982). In addition, among patients admitted to hospital for LRTIs, the admission rate of patients with diabetes (Winterbauer et al., 1969; Kornum et al., 2007; Peleg et al., 2007; Casqueiro et al., 2012), risk of complications (Peleg et al., 2007) and mortality (Fine et al., 1996; Kornum et al., 2007) were significantly higher than patients without diabetes. Double hit from an aging immune system, host defense may be impaired in diabetes together increase the risk of bacterial, mycobacterial, fungal and viral infections. Furthermore, respiratory dysfunction and microangiopathy together lead to a higher morbidity and mortality in diabetes elderly (Kornum et al., 2007).

Antimicrobial pharmacotherapy for elderly with diabetes is the same as for all age groups (Mandell et al., 2003). Data suggest that elderly patients receiving aminoglycosides have worse outcomes (Gleason et al., 1999), and medication regimen should be individualized, taking into account the patient's recent antibiotic medication history, comorbidities, suspected aspiration, suspected pseudomonas infection and β-lactam allergy. For pneumonia patients with diabetes, patients who have not recently used antibiotics can take advanced macrolides or a respiratory fluoroquinolone. By contrast, Patients who have used antibiotics recently can choose fluoroquinolone and advanced macrolides. The chronic use of inhaled glucocorticoids in elderly is associated with the increased risk of diabetes, physicians should be aware of this in order to select those patients in whom the benefits will outweigh the risks (Battaglia et al., 2015). At the same time, it is also important for the management of blood glucose level in infected patients. Meanwhile, diabetic patients usually have varying degrees of impaired renal function, antibiotics with nephrotoxicity should be avoided.

Fungal Pneumonia

Pulmonary fungal infections can occur in old patients with normal or impaired immune function. The morbidity and mortality of fungal pneumonia among the elderly have increased significantly in recent years. The reason is the increase in patients with malignant tumors, as well as organ transplants or autoimmune diseases, resulting in an increase in patients with immunocompromise, leading to an increase in the incidence of fungal pneumonia (Limper et al., 2011; Chen et al., 2018).

Candida pneumonia is rare; in fact, the isolation of Candida from respiratory secretions is of no clinical significance in most cases (Chen et al., 2018). For immunecompetent pulmonary cryptococcosis hosts, fluconazole or itraconazole are recommended, while immunocompromised hosts are recommended to be treated with amphotericin B in combination with flucytosine, and then followed by fluconazole or itraconazole (Li et al., 2017). In patients with normal immune function, patients with pulmonary aspergillosis are recommended to inhale glucocorticoids and bronchodilators and leukotriene receptor antagonists (Denning et al., 2016), while immunocompromised patients with invasive pulmonary aspergillosis are advised to take oral fluconazole or itraconazole or intravenous amphotericin B (Blanchard et al., 2018). For elderly patients with immunodeficiency, intravenous caspofungin or micafungin is recommended, then followed by oral fluconazole or itraconazole (Bao et al., 2017), meanwhile oral administration of posaconazole at the beginning of treatment is another choice (Clark et al., 2015).

Respiratory HCoVs

Severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) are single-stranded, enveloped, positive-sense RNA viruses. Age and underlying disease are pivotal independent predictors of miscellaneous adverse outcomes in SARS (Chan et al., 2003). SARS cases were mainly seen in young healthy individuals, but patients over 60 years old have the highest mortality, whereas half of the cases of MERS-CoV infections occurred in individuals over the age of 50 (Chan et al., 2003; Assiri et al., 2013). There is no difference in treatment options between the elderly and other age groups. Currently, the most commonly prescribed antiviral regimens are ribavirin, IFNs and lopinavir/ritonavir (Morgenstern et al., 2005; Al-Tawfiq et al., 2014; Omrani et al., 2014).

Ribavirin is a nucleoside analogue with broad-spectrum antiviral activity by inhibiting viral RNA synthesis and mRNA capping (von Grotthuss et al., 2003). The efficacy of ribavirin alone or in combination with IFN-β for the treatment of SARS is inconsistent and controversial (Chu et al., 2004; Leong et al., 2004), and Canada announced a ban on ribavirin for the treatment of SARS due to the reported side effects and inadequate efficacy (Chiou et al., 2005). Lopinavir and ritonavir are protease inhibitors that may inhibit the 3C-like protease of MERS, they improve clinical outcome compared with ribavirin alone in SARS patients (Chan et al., 2006; Stockman et al., 2006). There are still no commercial vaccines available against MERS-CoV (Hart et al., 2014). Multiple vaccine candidates targeting the S protein, which is responsible for viral entry, have been developed, including subunit vaccines (Wang et al., 2015; Tai et al., 2017) recombinant vector vaccines (Kim et al., 2014; Gilbert and Warimwe, 2017), and DNA vaccines (Al-Amri et al., 2017; Chi et al., 2017). Other agents, such as mycophenolic acid (MPA), which prevent replication of viral RNA, have showed strong inhibition activity against MERS-CoV *in vitro* studies (Hart et al., 2014). In addition, passive immunotherapy using human plasma was also applied in the treatment of SARS and MERS (Arabi et al., 2015; Mair-Jenkins et al., 2015). Generally, corticosteroids are widely used along with ribavirin during SARS outbreaks (Lee et al., 2004). A variety of other agents, including antiviral peptides, monoclonal antibodies, cellular or viral protease inhibitor may be promising agents for vitro and/or animal models (Ohnuma et al., 2013; Tao et al., 2014; Agrawal et al., 2016; Zumla et al., 2016). But the efficacy in patients with SARS and MERS needs further clinical validation. In *in vitro* experiments, IFN products were effective in inhibiting both SARS-CoV and MRES-CoV152 (Morgenstern et al., 2005; Chan et al., 2013). Meanwhile, although specific antivirals for MERS-CoV and SARS-CoV are developing, medication with repurposing potential, such as loperamide (de Wilde et al., 2014), chloroquine (Keyaerts et al., 2004), cyclophilins (Stamnes et al., 1992), kinase inhibitors (Dyall et al., 2014), may present as additional therapeutics for future coronaviruses.

Influenza

Influenza-related deaths gradually increase with increasing age (Yu et al., 2013). From 1979 to 2001, adults ≥65 years old accounted for approximately 60% of influenza-related hospitalizations (Casey et al., 2010; Nicoll, 2010). Data from central and south America (Cheng et al., 2015), European Centre for Disease Prevention and Control (ECDC) - Surveillance and Communication Unit (2011), Africa (Cohen et al., 2018), and southeast Asia (Wong et al., 2006; Park et al., 2016; Ang et al., 2017) are consistent, reporting higher morbidity and mortality in old adults.

Due to doubts about the potency of influenza vaccines, the vaccination rate of influenza vaccine among the elderly is very low (Schmid et al., 2017). In addition, insufficient supply of vaccine and vaccine hesitancy also contribute to inadequate vaccination for the elderly.

Some standard-dose (SD) influenza vaccine studies among elderly have estimated benefits in preventing hospitalization and mortality due to pneumonia (Nichol et al., 2003; Nichol et al., 2007; Jansen et al., 2008). Meanwhile, the high-dose (HD) trivalent influenza vaccine (TIV) was 22% more effective than SD influenza vaccine at preventing probable influenza infections, and 22% more effective than SD influenza vaccine in preventing influenza hospital admission (Izurieta et al., 2015). Another retrospective cohort of U.S. veterans found that, in the 85-year-old group, there was a significant reduction in hospitalizations influenza and pneumonia associated with the HD TIV injection (Richardson et al., 2015). According to observational studies and RCTs, HD TIV (Wong et al., 2006; Park et al., 2016), MF-59-adjuvanted influenza vaccine appeared to have efficacy for clinical influenza (i.e., ILI) and serologically confirmed influenza in adults older than 60 years old (Govaert et al., 1994; Engler et al., 2008; Taylor et al., 2012; Van Buynder et al., 2013; Darvishian et al., 2017; Domnich et al., 2017; Shay et al., 2017). For the diagnosed Influenza, neuraminidase inhibitors (NAI), including oseltamivir (Dobson et al., 2015), zanamivir (Heneghan et al., 2014), and peramivir (2015), are effective against both influenza A and influenza B viruses.

DISCUSSION

We reviewed a number of newly developed agents systematically, with the purpose to weigh their relative advantages and limitations for utilization in the elderly population. According to the key advantages, we classified the above antibiotics into "not recommended, moderate recommended and recommend". For example, telavancin's better bioavailability, food-independent efficacy, and simple dosing regimens that support once daily administration, make it a potential therapy for the elderly with LRTIs.

As for nemonoxacin, all the above trials of this medicine enrolled patients over the age of 65, while this age group had not been separated into a subgroup to test the safety and efficacy of certain antibiotics alone. But considering that the elderly accounts for the majority of the participants, we still recommended nemonoxacin as a first-line medication for LRTIs in elderly according to the key criteria we have formulated above.

By contrast, zabofloxaxin has little potential for the treatment of LRTIs in elderly. Although it can be a potential therapy for COPD patients with moderate-severity exacerbations, zabofloxacin is ineffective against common non-community acquired pathogens such as aeruginosa and *A. baumannii*. For elderly patients in long-term care centers or over-hospitalized patients with underlying diseases such as cystic fibrosis, it should be noted that zabofloxacin may not be applicable (Han et al., 2013). In addition, the safety and efficacy of intravenous formulation of zabofloxacin are still unclear. Moreover, zabofloxacin has not been approved by the Food and Drug Administration (FDA) for CAP treatment.

As for telavancin, it is a novel semi-synthetic lipoglycopeptides that is active against multidrug resistant (MDR) staphylococci, enterococci, and streptococci. Telavancin's better bioavailability, food-independent efficacy, and simple dosing regimens that support once daily administration, make it a potential therapy for old people with LRTIs.

Omadacycline was a novel once-daily aminomethylcycline antibiotic, and became the second tetracycline antibiotic approved by the FDA in 2018. Despite all the obvious advantages of omadacycline, enough attention should be given to the drawbacks for cardiac electrophysiology, namely, changes in heart rate (HR) and QT interval (Duraes and Sousa, 2019).

As a member of macrolides, solithromycin has little potential for the treatment of LRTIs in elderly. Based on the fact that solithromycin is an inhibitor of CYP3A4, the same caution should be used when co-administering solithromycin with agents that have demonstrated interaction with the precedent macrolides. Solithromycin appears to affect plasma concentrations of digoxin and warfarin, probably due to its interaction with P-glycoprotein and CYP3A4, leading to bradydysrhythmias and increased bleeding risk in elderly (Still et al., 2011). Sleep disorders and related medications, such as benzodiazepines, are commonly used in elderly, and these medications are mainly metabolized by CYP3A4 and induce side effects or attenuate therapeutic effects (Kasper and Resinger, 2001). Therefore, the combination of the two categories of agents should be avoided, and if it's unavoidable, the dose of benzodiazepines should be reduced.

In 2010, ceftaroline was approved by the FDA and European Medicines Agency (EMA) for the treatment of CAP. Ceftaroline is only recommended in the intravenous formation in a hospitalized setting for elderly CAP patients with creatinine clearance of ≥ > 30 mL/min, no QT prolongation history, and PORT risk classes III–IV. First of all, the lack of an oral formulation for ceftaroline is a limiting property for its use in the hospital setting. Secondly, ceftaroline has weak antibacterial activity against *E. faecium*, *VRE*, *ESBL-E*, and *P. aeruginosa* (Kiang et al., 2015), which are common pathogens found in HAP patients with comorbidities or long-term nursing homes and are frequently treated with antibiotics. In addition, although the AEs are mostly mild, in FOCUS 1, 1.4% of ceftaroline patients and 1.0% of ceftriaxone patients developed QTcB prolongation, both of which were >500 ms, with the elongation of ≥ > 60 ms compared with the baseline.

For another Cephalosporins, ceftobiprole is suitable for patients with suspected pneumonia caused by *MRSA*, *Enterobacteriaceae* or *P. aeruginosa*, especially for patients who live in nursing homes for a long time, but more data in elderly population are required for further recommendation. Ceftobiprole, q8h, IV limits the daily activities of the elderly. For old people with malnutrition and impaired cognitive function, the risk of sarcopenia, delirium, pressure ulcer, and sputum may be increased. A survey of about one-third of clinical trial centers found that a large portion of the data in these centers were unreliable or unverifiable (Abbas et al., 2017; Jean et al., 2017), thus the FDA has requested more information and recommended additional clinical studies before ceftobiprole is approved for cSSSI and pneumonia.

We have summarized the key advantages of lefamulin in treatment of LRTIs above. In our opinion, lefamulin should be recommended as a promising agent for LRTIs in elderly, and attention must be paid to its interaction with other medicines, such as azole antifungals (Paukner et al., 2019) and midazolam (File et al., 2019) at the same time. Beyond that, it takes 12 h to the intravenous use of lefamulin, which more or less limits the activity of elderly patients and increases the possibility of convulsions.

Until now, there is insufficient evidence to support tedizolid as an ideal antibiotic therapy for LRTIs in elderly at present. Tedizolid is still not a FDA/EMA-approved antibiotic for the treatment of LRTIs, but it does bring hope to patients suffering liver and kidney organ failure, especially for LRTIs associated with linezolid-resistant Gram-positive pathogens. Although the phase 3 trial (NCT02019420) of tedizolid for HAP has not yet yielded results, tedizolid brings hope to old patients suffering renal or hepatic failure, especially with linezolid-resistant Gram-positive pathogens pneumonia (Flanagan et al., 2018).

CONCLUSIONS

Despite noteworthy decreases in the number of deaths due to LRTIs, there remains an urgent need to make efforts to reduce the burden of disease in the elderly, especially for those with physical decline, mechanical ventilation, immunosuppression, frailty, dementia, and comorbidities. Although there are no pharmacotherapy and guidelines specifically for old patients with LRTIs, pharmacists and clinicians will need to weigh their various advantages and limitations based on the typical challenges that are faced by the elderly before choosing the optimal pharmacotherapy.

AUTHOR CONTRIBUTIONS

YL and BD conceived and designed the project. YL, YZ, WZ has contributed significantly to the submitted work and wrote the first draft. YL, XL, and FH revised the manuscript. All authors read and approved the final manuscript.

REFERENCES

(2015). Peramivir (Rapivab): an IV neuraminidase inhibitor for treatment of influenza. *Med. Lett. Drugs Ther.* 57 (1461), 17–19.

(2019). Omadacycline (Nuzyra)–a new tetracycline antibiotic. *JAMA* 322 (5), 457–458. doi: 10.1001/jama.2019.8199

3Rd, H. E., Golden, M., Jamieson, B. D., Dixon, P. B., Harbison, H. S., Lowens, S., et al. (2015). A phase 2 trial of oral solithromycin 1200 mg or 1000 mg as single-dose oral therapy for uncomplicated gonorrhea. *Clin. Infect. Dis.* 61 (7), 1043. doi: 10.1093/cid/civ478

Abbas, M., Paul, M., and Huttner, A. (2017). New and improved? A review of novel antibiotics for gram-positive bacteria. *Clin. Microbiol. Infect. Official Pub. Eur. Soc. Clin. Microbiol. Infect. Dis.* 23 (10), S1198743X17303294. doi: 10.1016/j.cmi.2017.06.010

Agrawal, A. S., Ying, T., Tao, X., Garron, T., Algaissi, A., Wang, Y., et al. (2016). Passive transfer of a germline-like neutralizing human monoclonal antibody protects transgenic mice against lethal middle east respiratory syndrome coronavirus infection. *Sci. Rep.* 6, 31629. doi: 10.1038/srep31629

Al-Amri, S. S., Abbas, A. T., Siddiq, L. A., Alghamdi, A., Sanki, M. A., Al-Muhanna, M. K., et al. (2017). Immunogenicity of candidate MERS-CoV DNA vaccines based on the spike protein. *Sci. Rep.* 7, 44875. doi: 10.1038/srep44875

Al-Tawfiq, J. A., Momattin, H., Dib, J., and Memish, Z. A. (2014). Ribavirin and interferon therapy in patients infected with the middle east respiratory syndrome coronavirus: an observational study. *Int. J. Infect. Dis.* 20, 42–46. doi: 10.1016/j.ijid.2013.12.003

Al Jalali, V., and Zeitlinger, M. (2018). Clinical pharmacokinetics and pharmacodynamics of telavancin compared with the other glycopeptides. *Clin. Pharmacokinet.* 57 (7), 797–816. doi: 10.1007/s40262-017-0623-4

Ales, T., Craig, R., Igor, G., Petr, H., Anna, S., Waleria, H., et al. (2013). Clinical and economic burden of community-acquired pneumonia among adults in the Czech Republic, Hungary, Poland and Slovakia. *Plos One* 8 (8), e71375. doi: 10.1371/journal.pone.0071375

Alldred, D. P., Standage, C., Zermansky, A. G., Barber, N. D., Raynor, D. K., and Petty, D. R. (2010). The recording of drug sensitivities for older people living in care homes. *Br. J. Clin. Pharmacol.* 69 (5), 553–557. doi: 10.1111/j.1365-2125.2010.03631.x

Ang, L. W., Yap, J., Lee, V., Chng, W. Q., Jaufeerally, F. R., Lam, C. S. P., et al. (2017). Influenza-associated hospitalizations for cardiovascular diseases in the tropics. *Am. J. Epidemiol.* 186 (2), 202–209. doi: 10.1093/aje/kwx001

Arabi, Y., Balkhy, H., Hajeer, A. H., Bouchama, A., Hayden, F. G., Al-Omari, A., et al. (2015). Feasibility, safety, clinical, and laboratory effects of convalescent plasma therapy for patients with middle east respiratory syndrome coronavirus infection: a study protocol. *Springerplus* 4, 709. doi: 10.1186/s40064-015-1490-9

Arnold, F. W., Summersgill, J. T., and Ramirez, J. A. (2016). Role of atypical pathogens in the etiology of community-acquired pneumonia. *Semin Respir. Crit. Care Med.* 37 (6), 819–828. doi: 10.1055/s-0036-1592121

Asche, C., McAdam-Marx, C., Seal, B., Crookston, B., and Mullins, C. D. (2008). Treatment costs associated with community-acquired pneumonia by community level of antimicrobial resistance. *J. Antimicrob. Chemother.* 61 (5), 1162–1168. doi: 10.1093/jac/dkn073

Assiri, A., Al-Tawfiq, J. A., Al-Rabeeah, A. A., Al-Rabiah, F. A., Al-Hajjar, S., Al-Barrak, A., et al. (2013). Epidemiological, demographic, and clinical characteristics of 47 cases of middle east respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect. Dis.* 13 (9), 752–761. doi: 10.1016/S1473-3099(13)70204-4

Awad, S. S., Rodriguez, A. H., Chuang, Y. C., Marjanek, Z., Pareigis, A. J., Reis, G., et al. (2014). A phase 3 randomized double-blind comparison of ceftobiprole medocaril versus ceftazidime plus linezolid for the treatment of hospital-acquired pneumonia. *Clin. Infect. Dis.* 59 (1), 51–61. doi: 10.1093/cid/ciu219

Baddour, L. M., Yu, V. L., Klugman, K. P., Feldman, C., Ortqvist, A., Rello, J., et al. (2004). Combination antibiotic therapy lowers mortality among severely ill patients with pneumococcal bacteremia. *Am. J. Respir. Crit. Care Med.* 170 (4), 440–444. doi: 10.1164/rccm.200311-1578OC

Bao, Z., Chen, H., Zhou, M., Shi, G., Li, Q., and Wan, H. (2017). Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: a case report and review of the literature. *Oncotarget* 8 (23), 38069–38074. doi: 10.18632/oncotarget.16971

Barrera, C. M., Mykietiuk, A., Metev, H., Nitu, M. F., Karimjee, N., Doreski, P. A., et al. (2016). Efficacy and safety of oral solithromycin versus oral moxifloxacin for treatment of community-acquired bacterial pneumonia: a global, double-blind, multicentre, randomised, active-controlled, non-inferiority trial (SOLITAIRE-ORAL). *Lancet Infect. Dis.* 16 (4), 421–430. doi: 10.1016/S1473-3099(16)00017-7

Barriere, S. L. (2014). The ATTAIN trials: efficacy and safety of telavancin compared with vancomycin for the treatment of hospital-acquired and ventilator-associated bacterial pneumonia. *Future Microbiol.* 9 (3), 281–289. doi: 10.2217/fmb.14.4

Bassetti, M., Vena, A., Croxatto, A., Righi, E., and Guery, B. (2018). How to manage Pseudomonas aeruginosa infections. *Drugs Context* 7, 212527. doi: 10.7573/dic.212527

Battaglia, S., Cardillo, I., Lavorini, F., Spatafora, M., and Scichilone, N. (2015). Erratum to: safety considerations of inhaled corticosteroids in the elderly. *Drugs Aging* 32 (12), 1067–1076. doi: 10.1007/s40266-015-0320-7

Bernardo, K., Pakulat, N., Fleer, S., Schnaith, A., Utermohlen, O., Krut, O., et al. (2004). Subinhibitory concentrations of linezolid reduce Staphylococcus aureus virulence factor expression. *Antimicrob. Agents Chemother.* 48 (2), 546–555. doi: 10.1128/AAC.48.2.546-555.2004

Blanchard, E., Gabriel, F., Jeanne-Leroyer, C., Servant, V., and Dumas, P. Y. (2018). [Invasive pulmonary aspergillosis]. *Rev. Mal. Respir.* 35 (2), 171–187. doi: 10.1016/j.rmr.2018.01.002

Blazquez Garrido, R. M., Espinosa Parra, F. J., Alemany Frances, L., Ramos Guevara, R. M., Sanchez-Nieto, J. M., Segovia Hernandez, M., et al. (2005). Antimicrobial chemotherapy for Legionnaires disease: levofloxacin versus macrolides. *Clin. Infect. Dis.* 40 (6), 800–806. doi: 10.1086/428049

Boyd, A. R., and Orihuela, C. J. (2011). Dysregulated inflammation as a risk factor for pneumonia in the elderly. *Aging Dis.* 2 (6), 487–500.

Bradley, J. S. (2014). Which antibiotic for resistant gram-positives, and why? *J. Infect.* 68 Suppl 1, S63–S75. doi: 10.1016/j.jinf.2013.09.016

Brown, S. D., and Traczewski, M. M. (2010). Comparative *in vitro* antimicrobial activities of torezolid (TR-700), the active moiety of a new oxazolidinone, torezolid phosphate (TR-701), determination of tentative disk diffusion interpretive criteria, and quality control ranges. *Antimicrob. Agents Chemother.* 54 (5), 2063–2069. doi: 10.1128/AAC.01569-09

Cao, B., Qu, J. X., Yin, Y. D., and Eldere, J. V. (2017). Overview of antimicrobial options for mycoplasma pneumoniae pneumonia: focus on macrolide resistance. *Clin. Respir. J.* 11 (4), 419–429. doi: 10.1111/crj.12379

Cao, G. Y., Zhang, J., Zhang, Y. Y., Guo, B. N., Yu, J. C., Wu, X. J., et al. (2014). Safety, tolerability, and pharmacokinetics of intravenous nemonoxacin in healthy chinese volunteers. *Antimicrob. Agents Chemother.* 58 (10), 6116–6121. doi: 10.1128/AAC.02972-14

Casey, C. G., Lyss, S. C., Gunn, R. A., Rutledge, T. F., Weatherwax, D. W., and Meadows, D. G. (2010). Estimates of deaths associated with seasonal influenza — United States, 1976-2007. *MMWR Morb. Mortal Wkly Rep.* 59 (33), 1057–1062.

Casqueiro, J., Casqueiro, J., and Alves, C. (2012). Infections in patients with diabetes mellitus: a review of pathogenesis. *Indian J. Endocrinol. Metab.* 16 Suppl 1, S27–S36. doi: 10.4103/2230-8210.94253

Chan, J. F., Chan, K. H., Kao, R. Y., To, K. K., Zheng, B. J., Li, C. P., et al. (2013). Broad-spectrum antivirals for the emerging middle east respiratory syndrome coronavirus. *J. Infect.* 67 (6), 606–616. doi: 10.1016/j.jinf.2013.09.029

Chan, K. S., Zheng, J. P., Mok, Y. W., Li, Y. M., Liu, Y. N., Chu, C. M., et al. (2003). SARS: prognosis, outcome and sequelae. *Respirology* 8 Suppl, S36–S40. doi: 10.1046/j.1440-1843.2003.00522.x

Chan, P. K., Tang, J. W., and Hui, D. S. (2006). SARS: clinical presentation, transmission, pathogenesis and treatment options. *Clin. Sci. (Lond.)* 110 (2), 193–204. doi: 10.1042/CS20050188

Chang, S. P., Lee, H. Z., Lai, C. C., and Tang, H. J. (2019). The efficacy and safety of nemonoxacin compared with levofloxacin in the treatment of community-acquired pneumonia: a systemic review and meta-analysis of randomized controlled trials. *Infect. Drug Resist. Volume* 12, 433–438. doi: 10.2147/IDR.S193233

Chen, M., Xu, Y., Hong, N., Yang, Y., Lei, W., Du, L., et al. (2018). Epidemiology of fungal infections in China. *Front. Med.* 12 (1), 58–75. doi: 10.1007/s11684-017-0601-0

Cheng, P. Y., Palekar, R., Azziz-Baumgartner, E., Iuliano, D., Alencar, A. P., Bresee, J., et al. (2015). Burden of influenza-associated deaths in the Americas, 2002-2008. *Influenza Other Respir. Viruses* 9 Suppl 1, 13–21. doi: 10.1111/irv.12317

Chi, H., Zheng, X., Wang, X., Wang, C., Wang, H., Gai, W., et al. (2017). DNA vaccine encoding middle east respiratory syndrome coronavirus S1 protein induces protective immune responses in mice. *Vaccine* 35 (16), 2069–2075. doi: 10.1016/j.vaccine.2017.02.063

Chiou, H. E., Liu, C. L., Buttrey, M. J., Kuo, H. P., Liu, H. W., Kuo, H. T., et al. (2005). Adverse effects of ribavirin and outcome in severe acute respiratory syndrome: experience in two medical centers. *Chest* 128 (1), 263–272. doi: 10.1378/chest.128.1.263

Choi, J. Y., Kim, S. W., Yoon, S. J., Kang, M. G., Kim, K. I., and Kim, C. H. (2018). Impact of frailty on do-not-resuscitate orders and healthcare transitions among elderly Koreans with pneumonia. *Clin. Interv. Aging* 13, 2237–2245. doi: 10.2147/CIA.S181400

Chu, C. M., Cheng, V. C., Hung, I. F., Wong, M. M., Chan, K. H., Chan, K. S., et al. (2004). Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* 59 (3), 252–256. doi: 10.1136/thorax.2003.012658

Clark, N. M., Grim, S. A., and Lynch, J. P. (2015). Posaconazole: use in the prophylaxis and treatment of fungal infections. *Semin Respir. Crit. Care Med.* 36 (5), 767–785. doi: 10.1055/s-0035-1562902

Cohen, C., Walaza, S., Treurnicht, F. K., McMorrow, M., Madhi, S. A., McAnerney, J. M., et al. (2018). In- and out-of-hospital mortality associated with seasonal and pandemic influenza and respiratory syncytial virus in South Africa, 2009-2013. *Clin. Infect. Dis.* 66 (1), 95–103. doi: 10.1093/cid/cix740

Cooper, E. C., Curtis, N., Cranswick, N., and Gwee, A. (2014). Pristinamycin: old drug, new tricks? *J. Antimicrob. Chemother.* 69 (9), 2319–2325. doi: 10.1093/jac/dku167

Curcio, D. (2014). Multidrug-resistant gram-negative bacterial infections: are you ready for the challenge? *Curr. Clin. Pharmacol.* 9 (1), 27–38. doi: 10.2174/15748847113089990062

Darpo, B., Sager, P. T., Fernandes, P., Jamieson, B. D., Keedy, K., Zhou, M., et al. (2017a). Solithromycin, a novel macrolide, does not prolong cardiac repolarization: a randomized, three-way crossover study in healthy subjects. *J. Antimicrob. Chemother.* 72 (2), 515. doi: 10.1093/jac/dkw428

Darpo, B., Sager, P. T., Fernandes, P., Jamieson, B. D., Keedy, K., Zhou, M., et al. (2017b). Solithromycin, a novel macrolide, does not prolong cardiac repolarization: a randomized, three-way crossover study in healthy subjects. *J. Antimicrob. Chemother.* 72 (2), 515–521. doi: 10.1093/jac/dkw428

Darvishian, M., van den Heuvel, E. R., Bissielo, A., Castilla, J., Cohen, C., Englund, H., et al. (2017). Effectiveness of seasonal influenza vaccination in community-dwelling elderly people: an individual participant data meta-analysis of test-negative design case-control studies. *Lancet Respir. Med.* 5 (3), 200–211. doi: 10.1016/S2213-2600(17)30043-7

Das, B., Sarkar, C., Das, D., Gupta, A., Kalra, A., and Sahni, S. (2017). Telavancin: a novel semisynthetic lipoglycopeptide agent to counter the challenge of resistant Gram-positive pathogens. *Ther. Adv. Infect. Dis.* 4 (2), 49–73. doi: 10.1177/2049936117690501

de Groot, R. C. A., Meyer Sauteur, P. M., Unger, W. W. J., and van Rossum, A. M. C. (2017). Things that could be Mycoplasma pneumoniae. *J. Infect.* 74 Suppl 1, S95–s100. doi: 10.1016/S0163-4453(17)30198-6

de Wilde, A. H., Jochmans, D., Posthuma, C. C., Zevenhoven-Dobbe, J. C., van Nieuwkoop, S., Bestebroer, T. M., et al. (2014). Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob. Agents Chemother.* 58 (8), 4875–4884. doi: 10.1128/AAC.03011-14

Denning, D. W., Cadranel, J., Beigelman-Aubry, C., Ader, F., Chakrabarti, A., Blot, S., et al. (2016). Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management. *Eur. Respir. J.* 47 (1), 45–68. doi: 10.1183/13993003.00583-2015

Ding, C., Yang, Z., Wang, J., Liu, X., Cao, Y., Pan, Y., et al. (2016). Prevalence of Pseudomonas aeruginosa and antimicrobial-resistant Pseudomonas aeruginosa in patients with pneumonia in mainland China: a systematic review and meta-analysis. *Int. J. Infect. Dis.* 49, 119–128. doi: 10.1016/j.ijid.2016.06.014

Dobson, J., Whitley, R. J., Pocock, S., and Monto, A. S. (2015). Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. *Lancet* 385 (9979), 1729–1737. doi: 10.1016/S0140-6736(14)62449-1

Domnich, A., Arata, L., Amicizia, D., Puig-Barbera, J., Gasparini, R., and Panatto, D. (2017). Effectiveness of MF59-adjuvanted seasonal influenza vaccine in the elderly: a systematic review and meta-analysis. *Vaccine* 35 (4), 513–520. doi: 10.1016/j.vaccine.2016.12.011

Dougherty, J. A., Sucher, A. J., Chahine, E. B., and Shihadeh, K. C. (2019). Omadacycline: A New Tetracycline Antibiotic. *Ann. Pharmacother.* 53 (5), 486–500. doi: 10.1177/1060028018818094

Duncan, L. R., Sader, H. S., Smart, J. I., Flamm, R. K., and Mendes, R. E. (2017). Telavancin activity *in vitro* tested against a worldwide collection of Gram-positive clinical isolates (2014). *J. Glob. Antimicrob. Resist.* 10, S2213716517301248. doi: 10.1016/j.jgar.2017.03.018

Duraes, F., and Sousa, E. (2019). Omadacycline: a newly approved antibacterial from the class of tetracyclines. *Pharmaceuticals (Basel)* 12 (2), E63. doi: 10.3390/ph12020063

Dyall, J., Coleman, C. M., Hart, B. J., Venkataraman, T., Holbrook, M. R., Kindrachuk, J., et al. (2014). Repurposing of clinically developed drugs for treatment of middle east respiratory syndrome coronavirus infection. *Antimicrob. Agents Chemother.* 58 (8), 4885–4893. doi: 10.1128/AAC.03036-14

Edelstein, P. H., Edelstein, M. A., Lehr, K. H., and Ren, J. (1996). In-vitro activity of levofloxacin against clinical isolates of Legionella spp, its pharmacokinetics in guinea pigs, and use in experimental Legionella pneumophila pneumonia. *J. Antimicrob. Chemother.* 37 (1), 117–126. doi: 10.1093/jac/37.1.117

Engler, R. J., Nelson, M. R., Klote, M. M., VanRaden, M. J., Huang, C. Y., Cox, N. J., et al. (2008). Half- vs full-dose trivalent inactivated influenza vaccine (2004-2005): age, dose, and sex effects on immune responses. *Arch. Intern. Med.* 168 (22), 2405–2414. doi: 10.1001/archinternmed.2008.513

European Centre for Disease Prevention and Control (ECDC) - Surveillance and Communication Unit. (2011). European centre for disease prevention and control publishes annual epidemiological report 2011. *Eur. Surveill* 16 (45). doi: 10.1186/1475-2875-10-340

Falco, V., Burgos, J., and Almirante, B. (2018). Ceftobiprole medocaril for the treatment of community-acquired pneumonia. *Expert Opin. Pharmacother.* 19 (13), 1503–1509. doi: 10.1080/14656566.2018.1516749

Farrell, D. J., Flamm, R. K., Sader, H. S., and Jones, R. N. (2014). Ceftobiprole activity against over 60,000 clinical bacterial pathogens isolated in Europe, Turkey, and Israel from 2005 to 2010. *Antimicrob. Agents Chemother.* 58 (7), 3882–3888. doi: 10.1128/AAC.02465-14

File, T. M., Jr., Goldberg, L., Das, A., Sweeney, C., Saviski, J., Gelone, S. P., et al. (2019). Efficacy and safety of IV-to-oral lefamulin, a pleuromutilin antibiotic, for treatment of community-acquired bacterial pneumonia: the phase 3 LEAP 1 trial. *Clin. Infect. Dis.* doi: 10.1093/cid/ciz090

File, T. M., Jr., Low, D. E., Eckburg, P. B., Talbot, G. H., Friedland, H. D., Lee, J., et al. (2011). FOCUS 1: a randomized, double-blinded, multicentre, Phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. *J. Antimicrob. Chemother.* 66 Suppl 3, iii19–iii32. doi: 10.1093/jac/dkr096

File, T. M., Jr., Rewerska, B., Vucinic-Mihailovic, V., Gonong, J. R. V., Das, A. F., Keedy, K., et al. (2016). SOLITAIRE-IV: a randomized, double-blind, multicenter study comparing the efficacy and safety of intravenous-to-oral solithromycin to intravenous-to-oral moxifloxacin for treatment of community-acquired bacterial pneumonia. *Clin. Infect. Dis.* 63 (8), 1007–1016. doi: 10.1093/cid/ciw490

Fine, M. J., Smith, M. A., Carson, C. A., Mutha, S. S., Sankey, S. S., Weissfeld, L. A., et al. (1996). Prognosis and outcomes of patients with community-acquired pneumonia. a meta-analysis. *JAMA* 275 (2), 134–141. doi: 10.1001/jama.1996.03530260048030

Flanagan, S., Bartizal, K., Minassian, S. L., Fang, E., and Prokocimer, P. (2013). In vitro, *in vivo*, and clinical studies of tedizolid to assess the potential for peripheral or central monoamine oxidase interactions. *Antimicrob. Agents Chemother.* 57 (7), 3060–3066. doi: 10.1128/AAC.00431-13

Flanagan, S. D., Minassian, S. L., and Prokocimer, P. (2018). Pharmacokinetics, safety, and tolerability of tedizolid phosphate in elderly subjects. *Clin. Pharmacol. Drug Dev.* 7 (7), 788–794. doi: 10.1002/cpdd.426

Francois, B., Luyt, C. E., Stover, C. K., Brubaker, J. O., Chastre, J., and Jafri, H. S. (2017). New strategies targeting virulence factors of Staphylococcus aureus and Pseudomonas aeruginosa. *Semin Respir. Crit. Care Med.* 38 (3), 346–358. doi: 10.1055/s-0037-1602715

Genne, D., Siegrist, H. H., Humair, L., Janin-Jaquat, B., and de Torrente, A. (1997). Clarithromycin versus amoxicillin-clavulanic acid in the treatment of community-acquired pneumonia. *Eur. J. Clin. Microbiol. Infect. Dis.* 16 (11), 783–788. doi: 10.1007/BF01700406

Gilbert, S. C., and Warimwe, G. M. (2017). Rapid development of vaccines against emerging pathogens: the replication-deficient simian adenovirus platform technology. *Vaccine* 35 (35 Pt A), 4461–4464. doi: 10.1016/j.vaccine.2017.04.085

Giske, C. G., Monnet, D. L., Otto, C., and Yehuda, C. (2008). Clinical and economic impact of common multidrug-resistant gram-negative bacilli. *Antimicrob. Agents Chemother.* 52 (3), 813. doi: 10.1128/AAC.01169-07

Gleason, P. P., Meehan, T. P., Fine, J. M., Galusha, D. H., and Fine, M. J. (1999). Associations between initial antimicrobial therapy and medical outcomes for hospitalized elderly patients with pneumonia. *Arch. Intern. Med.* 159 (21), 2562–2572. doi: 10.1001/archinte.159.21.2562

Govaert, T. M., Thijs, C. T., Masurel, N., Sprenger, M. J., Dinant, G. J., and Knottnerus, J. A. (1994). The efficacy of influenza vaccination in elderly individuals. A randomized double-blind placebo-controlled trial. *JAMA* 272 (21), 1661–1665. doi: 10.1001/jama.1994.03520210045030

Green, K., Mcgeer, A., Rudnick, W., Pong-Porter, S., Patel, S. N., and Low, D. E. (2014). In vitro activity of new Cephalosporins vs Streptococcus pneumoniae from the Canadian bacterial surveillance network: 2008–2011. *Curr. Microbiol.* 69 (5), 763–767. doi: 10.1007/s00284-014-0622-8

Han, H., Kim, S. E., Shin, K. H., Lim, C., Lim, K. S., Yu, K. S., et al. (2013). Comparison of pharmacokinetics between new quinolone antibiotics: the zabofloxacin hydrochloride capsule and the zabofloxacin aspartate tablet. *Curr. Med. Res. Opin.* 29 (10), 1349–1355. doi: 10.1185/03007995.2013.825591

Haranaga, S., Tateyama, M., Higa, F., Miyagi, K., Akamine, M., Azuma, M., et al. (2007). Intravenous ciprofloxacin versus erythromycin in the treatment of Legionella pneumonia. *Intern. Med.* 46 (7), 353–357. doi: 10.2169/internalmedicine.46.6006

Hart, B. J., Dyall, J., Postnikova, E., Zhou, H., Kindrachuk, J., Johnson, R. F., et al. (2014). Interferon-beta and mycophenolic acid are potent inhibitors of middle east respiratory syndrome coronavirus in cell-based assays. *J. Gen. Virol.* 95 (Pt 3), 571–577. doi: 10.1099/vir.0.061911-0

Hassoun, A., Friedman, B., and Saravolatz, L. D. (2017). Telavancin in the recent hospital acquired and ventilator associated pneumonia (HAP/VAP) 2016 guidelines. *Clin. Infect. Dis.* 64 (11), 1633. doi: 10.1093/cid/cix272

Heneghan, C. J., Onakpoya, I., Thompson, M., Spencer, E. A., Jones, M., and Jefferson, T. (2014). Zanamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments. *BMJ* 348, g2547. doi: 10.1136/bmj.g2547

Hodille, E., Delouere, L., Bouveyron, C., Meugnier, H., Bes, M., Tristan, A., et al. (2017). In vitro activity of ceftobiprole on 440 Staphylococcus aureus strains isolated from bronchopulmonary infections. *Med. Mal. Infect.* 47 (2), 152–157. doi: 10.1016/j.medmal.2016.10.004

Hook, E.W.3rd, Golden, M., Jamieson, B. D., Dixon, P. B., Harbison, H. S., Lowens, S., et al. (2015). A phase 2 trial of oral solithromycin 1200 mg or 1000 mg as single-dose oral therapy for uncomplicated gonorrhea. *Clin. Infect. Dis.* 61 (7), 1043–1048. doi: 10.1093/cid/civ478

Horn, K. S., Danziger, L. H., Rodvold, K. A., and Glowacki, R. C. (2017). Pharmacokinetic drug evaluation of ceftobiprole for the treatment of MRSA. *Expert Opin. Drug Metab. Toxicol.* 13 (4), 463–472. doi: 10.1080/17425255.2017.1303481

Housman, S. T., Pope, J. S., Russomanno, J., Salerno, E., Shore, E., Kuti, J. L., et al. (2012). Pulmonary disposition of tedizolid following administration of once-daily oral 200-milligram tedizolid phosphate in healthy adult volunteers. *Antimicrob. Agents Chemother.* 56 (5), 2627–2634. doi: 10.1128/AAC.05354-11

Huang, D. B., File, T. M., Jr., Dryden, M., Corey, G. R., Torres, A., and Wilcox, M. H. (2018). Surveillance of iclaprim activity: In vitro susceptibility of gram-positive pathogens collected from 2012 to 2014 from the United States, Asia Pacific, Latin American and Europe. *Diagn. Microbiol. Infect. Dis.* 90 (4), 329–334. doi: 10.1016/j.diagmicrobio.2017.12.001

Huang, D. B., Hawser, S., Gemmell, C. G., and Sahm, D. F. (2017). In vitro activity of iclaprim against methicillin-resistant Staphylococcus aureus nonsusceptible to Daptomycin, Linezolid, or Vancomycin: a pilot study. *Can. J. Infect. Dis. Med. Microbiol.* 2017, 1–6. doi: 10.1155/2017/3948626

Huang, D. B., Magnet, S., De Angelis, S., Holland, T. L., File, T. M., Jr., Dryden, M., et al. (2019). Surveillance of iclaprim activity: *in vitro* susceptibility of gram-positive skin infection pathogens collected from 2015 to 2016 from North America and Europe. *Diagn. Microbiol. Infect. Dis.* 93 (2), 154–158. doi: 10.1016/j.diagmicrobio.2018.09.002

Izurieta, H. S., Thadani, N., and Shay, D. K. (2015). Corrections. Comparative effectiveness of high-dose versus standard-dose influenza vaccines in US residents aged 65 years and older from 2012 to 2013 using Medicare data: a retrospective cohort analysis. *Lancet Infect. Dis.* 15 (3), 263. doi: 10.1016/S1473-3099(15)70070-8

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., et al. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 392 (10159), 1789–1858. doi: 10.1016/s0140-6736(18)32279-7

Jansen, A. G., Sanders, E. A., Nichol, K. L., van Loon, A. M., Hoes, A. W., and Hak, E. (2008). Decline in influenza-associated mortality among Dutch elderly following the introduction of a nationwide vaccination program. *Vaccine* 26 (44), 5567–5574. doi: 10.1016/j.vaccine.2008.08.003

Jean, S. S., Hsueh, P. R., Lee, W. S., Chang, H. T., Chou, M. Y., Chen, I. S., et al. (2009). Nationwide surveillance of antimicrobial resistance among Haemophilus influenzae and Streptococcus pneumoniae in intensive care units in Taiwan. *Eur. J. Clin. Microbiol. Infect. Dis.* 28 (8), 1013–1017. doi: 10.1007/s10096-009-0727-3

Jean, S. S., Lee, W. S., Yu, K. W., Liao, C. H., Hsu, C. W., Chang, F. Y., et al. (2017). Corrigendum to "Rates of susceptibility of carbapenems, ceftobiprole, and colistin against clinically important bacteria collected from intensive care units in 2007: results from the surveillance of multicenter antimicrobial resistance in Taiwan (SMART)". *J. Microbiol. Immunol. Infect.* 51(3), 423–424.

Jones, R. N., Sader, H. S., Moet, G. J., and Farrell, D. J. (2010). Declining antimicrobial susceptibility of Streptococcus pneumoniae in the United States: report from the SENTRY antimicrobial surveillance program (1998-2009). *Diagn. Microbiol. Infect. Dis.* 68 (3), 334–336. doi: 10.1016/j.diagmicrobio.2010.08.024

Justo, J. A., Mayer, S. M., Pai, M. P., Soriano, M. M., Danziger, L. H., Novak, R. M., et al. (2015). Pharmacokinetics of ceftaroline in normal body weight and obese (classes I, II, and III) healthy adult subjects. *Antimicrob. Agents Chemother.* 59 (7), 3956–3965. doi: 10.1128/AAC.00498-15

Kalil, A. C., Klompas, M., Haynatzki, G., and Rupp, M. E. (2013). Treatment of hospital-acquired pneumonia with linezolid or vancomycin: a systematic review and meta-analysis. *BMJ Open* 3 (10), e003912. doi: 10.1136/bmjopen-2013-003912

Kasper, S., and Resinger, E. (2001). Panic disorder: the place of benzodiazepines and selective serotonin reuptake inhibitors. *Eur. Neuropsychopharmacol.* 11 (4), 307–321. doi: 10.1016/S0924-977X(01)00100-6

Katzan, I. L., Cebul, R. D., Husak, S. H., Dawson, N. V., and Baker, D. W. (2003). The effect of pneumonia on mortality among patients hospitalized for acute stroke. *Neurology* 60 (4), 620–625. doi: 10.1212/01.WNL.0000046586.38284.60

Keyaerts, E., Vijgen, L., Maes, P., Neyts, J., and Van Ranst, M. (2004). In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem. Biophys. Res. Commun.* 323 (1), 264–268. doi: 10.1016/j.bbrc.2004.08.085

Kiang, T. K., Wilby, K. J., and Ensom, M. H. (2015). A critical review on the clinical pharmacokinetics, pharmacodynamics, and clinical trials of ceftaroline. *Clin. Pharmacokinet.* 54 (9), 915–931. doi: 10.1007/s40262-015-0281-3

Kim, E., Okada, K., Kenniston, T., Raj, V. S., AlHajri, M. M., Farag, E. A., et al. (2014). Immunogenicity of an adenoviral-based middle east respiratory syndrome coronavirus vaccine in BALB/c mice. *Vaccine* 32 (45), 5975–5982. doi: 10.1016/j.vaccine.2014.08.058

Kocsis, B., Domokos, J., and Szabo, D. (2016). Chemical structure and pharmacokinetics of novel quinolone agents represented by avarofloxacin, delafloxacin, finafloxacin, zabofloxacin and nemonoxacin. *Ann. Clin. Microbiol. Antimicrob.* 15 (1), 34. doi: 10.1186/s12941-016-0150-4

Kollef, M. H., and Betthauser, K. D. (2019). New antibiotics for community-acquired pneumonia. *Curr. Opin. Infect. Dis.* 32 (2), 169–175. doi: 10.1097/QCO.0000000000000526

Kornum, J. B., Thomsen, R. W., Riis, A., Lervang, H. H., Schonheyder, H. C., and Sorensen, H. T. (2007). Type 2 diabetes and pneumonia outcomes: a population-based cohort study. *Diabetes Care* 30 (9), 2251–2257. doi: 10.2337/dc06-2417

Kresken, M., Korber-Irrgang, B., Lauffer, J., Decker-Burgard, S., and Davies, T. (2011). In vitro activities of ceftobiprole combined with amikacin or

levofloxacin against Pseudomonas aeruginosa: evidence of a synergistic effect using time-kill methodology. *Int. J. Antimicrob. Agents* 38 (1), 70–75. doi: 10.1016/j.ijantimicag.2011.01.028

Kuo, S. C., Chen, P. C., Shiau, Y. R., Wang, H. Y., Lai, J. F. S., Huang, W., et al. (2014). Levofloxacin-resistant haemophilus influenzae, Taiwan, 2004-2010. *Emerg. Infect. Dis.* 20 (8), 1386–1390. doi: 10.3201/eid2008.140341

Kwon, A. R., Min, Y. H., Ryu, J. M., Choi, D. R., Shim, M. J., and Choi, E. C. (2006). In vitro and *in vivo* activities of DW-224a, a novel fluoroquinolone antibiotic agent. *J. Antimicrob. Chemother.* 58 (3), 684–688. doi: 10.1093/jac/dkl304

Lau, Y. J., Hsueh, P. R., Liu, Y. C., Shyr, J. M., Huang, W. K., Teng, L. J., et al. (2006). Comparison of *in vitro* activities of tigecycline with other antimicrobial agents against Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis in Taiwan. *Microb. Drug Resist.* 12 (2), 130–135. doi: 10.1089/mdr.2006.12.130

Laue, H., Weiss, L., Bernardi, A., Hawser, S., Lociuro, S., and Islam, K. (2007). In vitro activity of the novel diaminopyrimidine, iclaprim, in combination with folate inhibitors and other antimicrobials with different mechanisms of action. *J. Antimicrob. Chemother.* 60 (6), 1391–1394. doi: 10.1093/jac/dkm409

Lee, N., Allen Chan, K. C., Hui, D. S., Ng, E. K., Wu, A., Chiu, R. W., et al. (2004). Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. *J. Clin. Virol.* 31 (4), 304–309. doi: 10.1016/j.jcv.2004.07.006

Leong, H. N., Ang, B., Earnest, A., Teoh, C., Xu, W., and Leo, Y. S. (2004). Investigational use of ribavirin in the treatment of severe acute respiratory syndrome, Singapore, 2003. *Trop. Med. Int. Health* 9 (8), 923–927. doi: 10.1111/j.1365-3156.2004.01281.x

Li, X. Z., Plesiat, P., and Nikaido, H. (2015). The challenge of efflux-mediated antibiotic resistance in gram-negative bacteria. *Clin. Microbiol. Rev.* 28 (2), 337–418. doi: 10.1128/CMR.00117-14

Li, Y., Fang, W., Jiang, W., Hagen, F., Liu, J., Zhang, L., et al. (2017). Cryptococcosis in patients with diabetes mellitus II in mainland China: 1993-2015. *Mycoses* 60 (11), 706–713. doi: 10.1111/myc.12645

Liang, W., Chen, Y. C., Cao, Y. R., Liu, X. F., Huang, J., Hu, J. L., et al. (2013). Pharmacokinetics and pharmacodynamics of nemonoxacin against Streptococcus pneumoniae in an *in vitro* infection model. *Antimicrob. Agents Chemother.* 57 (7), 2942–2947. doi: 10.1128/AAC.01098-12

Limper, A. H., Knox, K. S., Sarosi, G. A., Ampel, N. M., Bennett, J. E., Catanzaro, A., et al. (2011). An official American Thoracic Society statement: treatment of fungal infections in adult pulmonary and critical care patients. *Am. J. Respir. Crit. Care Med.* 183 (1), 96–128. doi: 10.1164/rccm.2008-740ST

Lodise, T. P., Bidell, M. R., Flanagan, S. D., Zasowski, E. J., Minassian, S. L., and Prokocimer, P. (2016). Characterization of the haematological profile of 21 days of tedizolid in healthy subjects. *J. Antimicrob. Chemother.* 71 (9), 2553–2558. doi: 10.1093/jac/dkw206

Low, D. E., File, T. M., Jr., Eckburg, P. B., Talbot, G. H., David Friedland, H., Lee, J., et al. (2011). FOCUS 2: a randomized, double-blinded, multicentre, Phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. *J. Antimicrob. Chemother.* 66 Suppl 3, iii33–iii44. doi: 10.1093/jac/dkr097

Luke, L., Li-Wen, C., Cheng-Yuan, T., Ching-Hung, H., Chung, D. T., Aronstein, W. S., et al. (2010). Dose escalation study of the safety, tolerability, and pharmacokinetics of nemonoxacin (TG-873870), a novel potent broad-spectrum nonfluorinated quinolone, in healthy volunteers. *Antimicrob. Agents Chemother.* 54 (1), 405–410. doi: 10.1128/AAC.00682-09

Ma, H. M., Yu, R. H., and Woo, J. (2013). Recurrent hospitalisation with pneumonia is associated with higher 1-year mortality in frail older people. *Intern. Med. J.* 43 (11), 1210–1215. doi: 10.1111/imj.12258

Mair-Jenkins, J., Saavedra-Campos, M., Baillie, J. K., Cleary, P., Khaw, F. M., Lim, W. S., et al. (2015). The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *J. Infect. Dis.* 211 (1), 80–90. doi: 10.1093/infdis/jiu396

Mandell, L. A., Bartlett, J. G., Dowell, S. F., File, T. M., Jr., Musher, D. M., and Whitney, C. (2003). Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. *Clin. Infect. Dis.* 37 (11), 1405–1433. doi: 10.1086/380488

Marchello, C., Dale, A. P., Thai, T. N., Han, D. S., and Ebell, M. H. (2016). Prevalence of atypical pathogens in patients with cough and community-acquired pneumonia: a meta-analysis. *Ann. Fam. Med.* 14 (6), 552–566. doi: 10.1370/afm.1993

Martinez, J. A., Horcajada, J. P., Almela, M., Marco, F., Soriano, A., Garcia, E., et al. (2003). Addition of a macrolide to a beta-lactam-based empirical antibiotic regimen is associated with lower in-hospital mortality for patients with bacteremic pneumococcal pneumonia. *Clin. Infect. Dis.* 36 (4), 389–395. doi: 10.1086/367541

Mendes, R. E., Paukner, S., Doyle, T. B., Gelone, S. P., Flamm, R. K., and Sader, H. S. (2019). Low prevalence of gram-positive isolates showing elevated lefamulin MIC results during the SENTRY surveillance program for 2015-2016 and characterization of resistance mechanisms. *Antimicrob. Agents Chemother.* 63 (4), e02158–18. doi: 10.1128/AAC.02158-18

Miller, A. C. (1981). Erythromycin in legionnaires' disease: a re-appraisal. *J. Antimicrob. Chemother.* 7 (3), 217–222. doi: 10.1093/jac/7.3.217

Mohamed, N. M., Zakaria, A. S., Edward, E. A., and Abdel-Bary, A. (2019). In vitro and in vivo activity of Zabofloxacin and other Fluoroquinolones against MRSA isolates from a University Hospital in Egypt. *Pol. J. Microbiol.* 68 (1), 59–69. doi: 10.21307/pjm-2019-007

Morgenstern, B., Michaelis, M., Baer, P. C., Doerr, H. W., and Cinatl, J., Jr. (2005). Ribavirin and interferon-beta synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines. *Biochem. Biophys. Res. Commun.* 326 (4), 905–908. doi: 10.1016/j.bbrc.2004.11.128

Mufson, M. A., and Stanek, R. J. (1999). Bacteremic pneumococcal pneumonia in one American City: a 20-year longitudinal study, 1978-1997. *Am. J. Med.* 107 (1a), 34s–43s. doi: 10.1016/S0002-9343(99)00098-4

Mykietiuk, A., Carratala, J., Fernandez-Sabe, N., Dorca, J., Verdaguer, R., Manresa, F., et al. (2005). Clinical outcomes for hospitalized patients with Legionella pneumonia in the antigenuria era: the influence of levofloxacin therapy. *Clin. Infect. Dis.* 40 (6), 794–799. doi: 10.1086/428059

Nespoulous, L., Matei, I., Charissoux, A., Bedane, C., and Assikar, S. (2018). Symmetrical drug-related intertriginous and flexural exanthema (SDRIFE) associated with pristinamycin, secnidazole, and nefopam, with a review of the literature. *Contact Dermat.* 79 (6), 378–380. doi: 10.1111/cod.13084

Nichol, K. L., Nordin, J., Mullooly, J., Lask, R., Fillbrandt, K., and Iwane, M. (2003). Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly. *N. Engl. J. Med.* 348 (14), 1322–1332. doi: 10.1056/NEJMoa025028

Nichol, K. L., Nordin, J. D., Nelson, D. B., Mullooly, J. P., and Hak, E. (2007). Effectiveness of influenza vaccine in the community-dwelling elderly. *N. Engl. J. Med.* 357 (14), 1373–1381. doi: 10.1056/NEJMoa070844

Nicholson, S. C., Welte, T., File, T. M., Jr., Strauss, R. S., Michiels, B., Kaul, P., et al. (2012). A randomised, double-blind trial comparing ceftobiprole medocaril with ceftriaxone with or without linezolid for the treatment of patients with community-acquired pneumonia requiring hospitalisation. *Int. J. Antimicrob. Agents* 39 (3), 240–246. doi: 10.1016/j.ijantimicag.2011.11.005

Nicoll, A. (2010). Pandemic risk prevention in European countries: role of the ECDC in preparing for pandemics. Development and experience with a national self-assessment procedure, 2005-2008. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 53 (12), 1267–1275. doi: 10.1007/s00103-010-1163-3

Ocheretyaner, E. R., and Park, T. E. (2018). Delafloxacin: a novel fluoroquinolone with activity against methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa. *Expert Rev. Anti Infect. Ther.* 16 (7), 523–530. doi: 10.1080/14787210.2018.1489721

Ohnuma, K., Haagmans, B. L., Hatano, R., Raj, V. S., Mou, H., Iwata, S., et al. (2013). Inhibition of middle east respiratory syndrome coronavirus infection by anti-CD26 monoclonal antibody. *J. Virol.* 87 (24), 13892–13899. doi: 10.1128/JVI.02448-13

Omrani, A. S., Saad, M. M., Baig, K., Bahloul, A., Abdul-Matin, M., Alaidaroos, A. Y., et al. (2014). Ribavirin and interferon alfa-2a for severe middle east respiratory syndrome coronavirus infection: a retrospective cohort study. *Lancet Infect. Dis.* 14 (11), 1090–1095. doi: 10.1016/S1473-3099(14)70920-X

Ortiz-Ruiz, G., Vetter, N., Isaacs, R., Carides, A., Woods, G. L., and Friedland, I. (2004). Ertapenem versus ceftriaxone for the treatment of community-acquired pneumonia in adults: combined analysis of two multicentre randomized, double-blind studies. *J. Antimicrob. Chemother.* 53 Suppl 2, ii59–ii66. doi: 10.1093/jac/dkh207

Park, H. S., Jung, S. J., Kwak, J. H., Choi, D. R., and Choi, E. C. (2010). DNA gyrase and topoisomerase IV are dual targets of zabofloxacin in Streptococcus

pneumoniae. *Int. J. Antimicrob. Agents* 36 (1), 97–98. doi: 10.1016/j.ijantimicag.2010.02.022

Park, H. S., Kim, H. J., Seol, M. J., Choi, D. R., Choi, E. C., and Kwak, J. H. (2006). In vitro and *in vivo* antibacterial activities of DW-224a, a new fluoronaphthyridone. *Antimicrob. Agents Chemother.* 50 (6), 2261–2264. doi: 10.1128/AAC.01407-05

Park, M., Wu, P., Goldstein, E., Joo Kim, W., and Cowling, B. J. (2016). Influenza-associated excess mortality in South Korea. *Am. J. Prev. Med.* 50 (4), e111–e119. doi: 10.1016/j.amepre.2015.09.028

Paukner, S., Gelone, S. P., Arends, S. J. R., Flamm, R. K., and Sader, H. S. (2019). Antibacterial activity of Lefamulin against pathogens most commonly causing community-acquired bacterial pneumonia: SENTRY antimicrobial surveillance program (2015-2016). *Antimicrob. Agents Chemother.* 63 (4), e02161–18. doi: 10.1128/AAC.02161-18

Peleg, A. Y., Weerarathna, T., McCarthy, J. S., and Davis, T. M. (2007). Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. *Diabetes Metab. Res. Rev.* 23 (1), 3–13. doi: 10.1002/dmrr.682

Perrone, T., and Quaglia, F. (2017). Lung US features of severe interstitial pneumonia: case report and review of the literature. *J. Ultrasound* 20 (3), 247–249. doi: 10.1007/s40477-017-0241-x

Peto, L., Nadjm, B., Horby, P., Ngan, T. T., van Doorn, R., Van Kinh, N., et al. (2014). The bacterial aetiology of adult community-acquired pneumonia in Asia: a systematic review. *Trans. R. Soc. Trop. Med. Hyg.* 108 (6), 326–337. doi: 10.1093/trstmh/tru058

Pfaller, M. A., Flamm, R. K., Mendes, R. E., Streit, J. M., Smart, J. I., Hamed, K. A., et al. (2019). Ceftobiprole activity against gram-positive and -negative pathogens collected from the United States in 2006 and 2016. *Antimicrob. Agents Chemother.* 63 (1), e01566–18. doi: 10.1128/AAC.01566-18

Pfaller, M. A., Huband, M. D., Rhomberg, P. R., and Flamm, R. K. (2017a). Surveillance of omadacycline activity against clinical isolates from a global collection (North America, Europe, Latin America, Asia-Western Pacific), 2010-2011. *Antimicrob. Agents Chemother.* 61 (5), e00018–17. doi: 10.1128/AAC.00018-17

Pfaller, M. A., Mendes, R. E., Castanheira, M., Flamm, R. K., Jones, R. N., and Sader, H. S. (2017b). Ceftaroline activity tested against bacterial isolates causing community-acquired respiratory tract infections and skin and skin structure infections in pediatric patients from United States Hospitals: 2012-2014. *Pediatr. Infect. Dis. J.* 36 (5), 486–491. doi: 10.1097/INF.0000000000001477

Pfaller, M. A., Sader, H. S., Rhomberg, P. R., and Flamm, R. K. (2017c). In vitro activity of delafloxacin against contemporary bacterial pathogens from the United States and Europe, 2014. *Antimicrob. Agents Chemother.* 61 (4), e02609–16. doi: 10.1128/AAC.02609-16

Pooley, N., Chadda, S., Madrigal, A. M., Kuessner, D., and Posthumus, J. (2014). A network meta-analysis comparing the efficacy and safety of Ceftobiprole and selected comparators in the treatment of hospital-acquired pneumonia. *Value Health* 17 (7), A588. doi: 10.1016/j.jval.2014.08.2012

Poulose, N., and Raju, R. (2014). Aging and injury: alterations in cellular energetics and organ function. *Aging Dis.* 5 (2), 101–108. doi: 10.14336/AD.2014.0500101

Rayfield, E. J., Ault, M. J., Keusch, G. T., Brothers, M. J., Nechemias, C., and Smith, H. (1982). Infection and diabetes: the case for glucose control. *Am. J. Med.* 72 (3), 439–450. doi: 10.1016/0002-9343(82)90511-3

Reid, A. B., Daffy, J. R., Stanley, P., and Buising, K. L. (2010). Use of pristinamycin for infections by gram-positive bacteria: clinical experience at an Australian hospital. *Antimicrob. Agents Chemother.* 54 (9), 3949–3952. doi: 10.1128/AAC.00212-10

Rhee, C. K., Chang, J. H., Choi, E. G., Kim, H. K., Kwon, Y. S., Kyung, S. Y., et al. (2015). Zabofloxacin versus moxifloxacin in patients with COPD exacerbation: a multicenter, double-blind, double-dummy, randomized, controlled, Phase III, non-inferiority trial. *Int. J. Chron. Obstruct. Pulmon. Dis.* 10, 2265–2275. doi: 10.2147/COPD.S90948

Richardson, D. M., Medvedeva, E. L., Roberts, C. B., and Linkin, D. R. (2015). Comparative effectiveness of high-dose versus standard-dose influenza vaccination in community-dwelling veterans. *Clin. Infect. Dis.* 61 (2), 171–176. doi: 10.1093/cid/civ261

Riquelme, S. A., Ahn, D., and Prince, A. (2018). Pseudomonas aeruginosa and Klebsiella pneumoniae adaptation to innate immune clearance mechanisms in the lung. *J. Innate. Immun.* 10 (5-6), 442–454. doi: 10.1159/000487515

Roblin, P. M., Kutlin, A., Sokolovskaya, N., and Hammerschlag, M. R. (1997). In-vitro activity of dirithromycin against Chlamydia pneumoniae. *J. Antimicrob. Chemother.* 39 (5), 647–649. doi: 10.1093/jac/39.5.647

Roychoudhury, S., Makin, K., Twinem, T., Leunk, R., and Hsu, M. C. (2016). In vitro resistance development to Nemonoxacin in Streptococcus pneumoniae: a unique profile for a novel nonfluorinated quinolone. *Microb. Drug Resist.* 22 (7), 578–584. doi: 10.1089/mdr.2016.0021

Rubinstein, E., Lalani, T., Corey, G. R., Kanafani, Z. A., Nannini, E. C., Rocha, M. G., et al. (2011). Telavancin versus vancomycin for hospital-acquired pneumonia due to gram-positive pathogens. *Clin. Infect. Dis.* 52 (1), 31–40. doi: 10.1093/cid/ciq031

Sabria, M., Pedro-Botet, M. L., Gomez, J., Roig, J., Vilaseca, B., Sopena, N., et al. (2005). Fluoroquinolones vs macrolides in the treatment of Legionnaires disease. *Chest* 128 (3), 1401–1405. doi: 10.1378/chest.128.3.1401

Scheeren, T. W. (2015). Ceftobiprole medocaril in the treatment of hospital-acquired pneumonia. *Future Microbiol.* 10 (12), 1913–1928. doi: 10.2217/fmb.15.115

Schmid, P., Rauber, D., Betsch, C., Lidolt, G., and Denker, M. L. (2017). Barriers of influenza vaccination intention and behavior - a systematic review of influenza vaccine hesitancy, 2005 - 2016. *PLoS One* 12 (1), e0170550. doi: 10.1371/journal.pone.0170550

Sharma, L., Losier, A., Tolbert, T., Dela Cruz, C. S., and Marion, C. R. (2017). Atypical pneumonia: updates on Legionella, Chlamydophila, and Mycoplasma Pneumonia. *Clin. Chest Med.* 38 (1), 45–58. doi: 10.1016/j.ccm.2016.11.011

Shay, D. K., Chillarige, Y., Kelman, J., Forshee, R. A., Foppa, I. M., Wernecke, M., et al. (2017). Comparative effectiveness of high-dose versus standard-dose influenza vaccines among US medicare beneficiaries in preventing Postinfluenza deaths during 2012-2013 and 2013-2014. *J. Infect. Dis.* 215 (4), 510–517. doi: 10.1093/infdis/jiw641

Siala, W., Kucharíková, S., Braem, A., Vleugels, J., Tulkens, P. M., Mingeot-Leclercq, M. P., et al. (2016). The antifungal caspofungin increases fluoroquinolone activity against Staphylococcus aureus biofilms by inhibiting N-acetylglucosamine transferase. *Nat. Commun.* 7, 13286. doi: 10.1038/ncomms13286

Song, W. J., and Chang, Y. S. (2017). Respiratory allergies in the elderly: findings from the Korean longitudinal study on health and aging phase I study (2005-2006). *Asia Pac. Allergy* 7 (4), 185–192. doi: 10.5415/apallergy.2017.7.4.185

Stamnes, M. A., Rutherford, S. L., and Zuker, C. S. (1992). Cyclophilins: a new family of proteins involved in intracellular folding. *Trends Cell Biol.* 2 (9), 272–276. doi: 10.1016/0962-8924(92)90200-7

Stets, R., Popescu, M., Gonong, J. R., Mitha, I., Nseir, W., Madej, A., et al. (2019). Omadacycline for community-acquired bacterial pneumonia. *N. Engl. J. Med.* 380 (6), 517–527. doi: 10.1056/NEJMoa1800201

Stevens, D. L., Ma, Y., Salmi, D. B., McIndoo, E., Wallace, R. J., and Bryant, A. E. (2007). Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant Staphylococcus aureus. *J. Infect. Dis.* 195 (2), 202–211. doi: 10.1086/510396

Still, J. G., Schranz, J., Degenhardt, T. P., Scott, D., Fernandes, P., Gutierrez, M. J., et al. (2011). Pharmacokinetics of solithromycin (CEM-101) after single or multiple oral doses and effects of food on single-dose bioavailability in healthy adult subjects. *Antimicrob. Agents Chemother.* 55 (5), 1997–2003. doi: 10.1128/AAC.01429-10

Stockman, L. J., Bellamy, R., and Garner, P. (2006). SARS: systematic review of treatment effects. *PLoS Med.* 3 (9), e343. doi: 10.1371/journal.pmed.0030343

Tai, W., Wang, Y., Fett, C. A., Zhao, G., Li, F., Perlman, S., et al. (2017). Recombinant receptor-binding domains of multiple middle east respiratory Syndrome Coronaviruses (MERS-CoVs) induce cross-neutralizing antibodies against divergent human and camel MERS-CoVs and antibody escape mutants. *J. Virol.* 91 (1), e01651–16. doi: 10.1128/JVI.01651-16

Tao, X., Mei, F., Agrawal, A., Peters, C. J., Ksiazek, T. G., Cheng, X., et al. (2014). Blocking of exchange proteins directly activated by cAMP leads to reduced replication of middle east respiratory syndrome coronavirus. *J. Virol.* 88 (7), 3902–3910. doi: 10.1128/JVI.03001-13

Taylor, D. N., Treanor, J. J., Sheldon, E. A., Johnson, C., Umlauf, S., Song, L., et al. (2012). Development of VAX128, a recombinant hemagglutinin (HA) influenza-flagellin fusion vaccine with improved safety and immune response. *Vaccine* 30 (39), 5761–5769. doi: 10.1016/j.vaccine.2012.06.086

Troeger, C., Forouzanfar, M., Rao, P. C., Khalil, I., Brown, A., Swartz, S., et al. (2017). Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect. Dis.* 17 (11), 1133–1161. doi: 10.1016/s1473-3099(17)30396-1

Van Buynder, P. G., Konrad, S., Van Buynder, J. L., Brodkin, E., Krajden, M., Ramler, G., et al. (2013). The comparative effectiveness of adjuvanted and unadjuvanted trivalent inactivated influenza vaccine (TIV) in the elderly. *Vaccine* 31 (51), 6122–6128. doi: 10.1016/j.vaccine.2013.07.059

van Rensburg, D. J., Perng, R. P., Mitha, I. H., Bester, A. J., Kasumba, J., Wu, R. G., et al. (2010). Efficacy and safety of nemonoxacin versus levofloxacin for community-acquired pneumonia. *Antimicrob. Agents Chemother.* 54 (10), 4098–4106. doi: 10.1128/AAC.00295-10

Varner, T. R., Bookstaver, P. B., Rudisill, C. N., and Albrecht, H. (2011). Role of rifampin-based combination therapy for severe community-acquired Legionella pneumophila pneumonia. *Ann. Pharmacother.* 45 (7-8), 967–976. doi: 10.1345/aph.1Q074

Vergis, E. N., Indorf, A., File, T. M., Jr., Phillips, J., Bates, J., Tan, J., et al. (2000). Azithromycin vs cefuroxime plus erythromycin for empirical treatment of community-acquired pneumonia in hospitalized patients: a prospective, randomized, multicenter trial. *Arch. Intern. Med.* 160 (9), 1294–1300. doi: 10.1001/archinte.160.9.1294

Veve, M. P., and Wagner, J. L. (2018). Lefamulin: Review of a Promising Novel Pleuromutilin Antibiotic. *Pharmacotherapy* 38 (9), 935–946. doi: 10.1002/phar.2166

von Grotthuss, M., Wyrwicz, L. S., and Rychlewski, L. (2003). mRNA cap-1 methyltransferase in the SARS genome. *Cell* 113 (6), 701–702. doi: 10.1016/S0092-8674(03)00424-0

Waites, K. B., Crabb, D. M., Duffy, L. B., Jensen, J. S., Liu, Y., and Paukner, S. (2017a). In vitro activities of Lefamulin and other antimicrobial agents against macrolide-susceptible and macrolide-resistant mycoplasma pneumoniae from the United States, Europe, and China. *Antimicrob. Agents Chemother.* 61 (2), e02008–16. doi: 10.1128/AAC.02008-16

Waites, K. B., Crabb, D. M., Liu, Y., and Duffy, L. B. (2016). In vitro activities of Omadacycline (PTK 0796) and other antimicrobial agents against human mycoplasmas and ureaplasmas. *Antimicrob. Agents Chemother.* 60 (12), e02008–16. doi: 10.1128/AAC.01734-16

Waites, K. B., Xiao, L., Liu, Y., Balish, M. F., and Atkinson, T. P. (2017b). Mycoplasma pneumoniae from the respiratory tract and beyond. *Clin. Microbiol. Rev.* 30 (3), 747–809. doi: 10.1128/CMR.00114-16

Wang, L., Shi, W., Joyce, M. G., Modjarrad, K., Zhang, Y., Leung, K., et al. (2015). Evaluation of candidate vaccine approaches for MERS-CoV. *Nat. Commun.* 6, 7712. doi: 10.1038/ncomms8712

Waterer, G. W., Somes, G. W., and Wunderink, R. G. (2001). Monotherapy may be suboptimal for severe bacteremic pneumococcal pneumonia. *Arch. Intern. Med.* 161 (15), 1837–1842. doi: 10.1001/archinte.161.15.1837

Webley, W. C., and Hahn, D. L. (2017). Infection-mediated asthma: etiology, mechanisms and treatment options, with focus on Chlamydia pneumoniae and macrolides. *Respir. Res.* 18 (1), 98. doi: 10.1186/s12931-017-0584-z

Winterbauer, R. H., Bedon, G. A., and Ball, W. C., Jr. (1969). Recurrent pneumonia. Predisposing illness and clinical patterns in 158 patients. *Ann. Intern. Med.* 70 (4), 689–700. doi: 10.7326/0003-4819-70-4-689

Wong, C. M., Yang, L., Chan, K. P., Leung, G. M., Chan, K. H., Guan, Y., et al. (2006). Influenza-associated hospitalization in a subtropical city. *PLoS Med.* 3 (4), e121. doi: 10.1371/journal.pmed.0030121

Woodhead, M., Blasi, F., Ewig, S., Garau, J., Huchon, G., Ieven, M., et al. (2011). Guidelines for the management of adult lower respiratory tract infections–summary. *Clin. Microbiol. Infect.* 17 (s6), E1–E59. doi: 10.1111/j.1469-0691.2011.03602.x

Wunderink, R. G., Rello, J., Cammarata, S. K., Croos-Dabrera, R. V., and Kollef, M. H. (2003). Linezolid vs vancomycin: analysis of two double-blind studies of patients with methicillin-resistant Staphylococcus aureus nosocomial pneumonia. *Chest* 124 (5), 1789–1797. doi: 10.1016/S0012-3692(15)33412-7

Yakovlev, S. V., Stratchounski, L. S., Woods, G. L., Adeyi, B., McCarroll, K. A., Ginanni, J. A., et al. (2006). Ertapenem versus cefepime for initial empirical treatment of pneumonia acquired in skilled-care facilities or in hospitals outside the intensive care unit. *Eur. J. Clin. Microbiol. Infect. Dis.* 25 (10), 633–641. doi: 10.1007/s10096-006-0193-0

Yu, H., Feng, L., Viboud, C. G., Shay, D. K., Jiang, Y., Zhou, H., et al. (2013). Regional variation in mortality impact of the 2009 A(H1N1) influenza pandemic in China. *Influenza Other Respir. Viruses* 7 (6), 1350–1360. doi: 10.1111/irv.12121

Yuan, J., Mo, B., Ma, Z., Lv, Y., Cheng, S. L., Yang, Y., et al. (2019). Safety and efficacy of oral nemonoxacin versus levofloxacin in treatment of community-acquired pneumonia: a phase 3, multicenter, randomized, double-blind, double-dummy, active-controlled, non-inferiority trial. *J. Microbiol. Immunol. Infect.* 52 (1), 35–44. doi: 10.1016/j.jmii.2017.07.011

Zhang, Y. F., Dai, X. J., Yang, Y., Chen, X. Y., Wang, T., Tang, Y. B., et al. (2016). Effects of probenecid and cimetidine on the pharmacokinetics of nemonoxacin in healthy Chinese volunteers. *Drug Des. Devel. Ther.* 10, 357–370. doi: 10.2147/DDDT.S95934

Zhong, N. S., Sun, T., Zhuo, C., D'Souza, G., Lee, S. H., Lan, N. H., et al. (2015). Ceftaroline fosamil versus ceftriaxone for the treatment of Asian patients with community-acquired pneumonia: a randomised, controlled, double-blind, phase 3, non-inferiority with nested superiority trial. *Lancet Infect. Dis.* 15 (2), 161–171. doi: 10.1016/S1473-3099(14)71018-7

Zmira, S., Orit, O., and Haim, S. (2005). Susceptibility of methicillin-resistant Staphylococcus aureus to vancomycin, teicoplanin, linezolid, pristinamycin and other antibiotics. *Israel Med. Assoc. J.* 7 (3), 148–150. doi:10.1007/s00108-004-1340-2

Zumla, A., Chan, J. F., Azhar, E. I., Hui, D. S., and Yuen, K. Y. (2016). Coronaviruses - drug discovery and therapeutic options. *Nat. Rev. Drug Discov.* 15 (5), 327–347. doi: 10.1038/nrd.2015.37

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Improvement of Sepsis Prognosis by Ulinastatin: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

Huifang Wang[1†], Bin Liu[2†], Ying Tang[1†], Ping Chang[1], Lishuai Yao[3], Bo Huang[1], Robert F. Lodato[4] and Zhanguo Liu[1]*

[1] Department of Intensive Care Unit, Zhujiang Hospital, Southern Medical University, Guangzhou, China, [2] Emergency Department, Zhujiang Hospital, Southern Medical University, Guangzhou, China, [3] Department of Thoracic and Cardiovascular Surgical, Zhujiang Hospital, Southern Medical University, Guangzhou, China, [4] Department of Pulmonary, Critical Care, and Sleep Medicine, Medical School, University of Texas Health Science Center at Houston, TX, United States

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***Correspondence:**
Zhanguo Liu
zhguoliu@163.com

†These authors have contributed equally to this work

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Background: Ulinastatin has been prescribed to treat sepsis. However, there is doubt regarding the extent of any improvement in outcomes to guide future decision making.

Objectives: To evaluate the effects of ulinastatin on mortality and related outcomes in sepsis patients.

Methods: Thirteen randomized controlled trials and two prospective studies published before September 1, 2018, that included 1358 patients with sepsis, severe sepsis, or septic shock were evaluated. The electronic databases searched in this study were PubMed, Medline, Embase, and China National Knowledge Infrastructure (CNKI) for Chinese Technical Periodicals.

Results: Ulinastatin significantly decreased the all-cause mortality {odds ratio (OR) = 0.48, 95% confidence interval (CI) [0.35, 0.66], $p < 0.00001$, $I^2 = 13\%$}, Acute Physiology, Age, Chronic Health Evaluation II (APACHE II) score {mean difference (MD) = -3.18, 95%CI [-4.01, -2.35], $p < 0.00001$, $I^2 = 33\%$, and reduced the incidence of multiple organ dysfunction syndrome (MODS) (OR = 0.3, 95% CI [0.18, 0.49], $p < 0.00001$, $I^2 = 0\%$). Ulinastatin also decreased the serum levels of IL-6 (MD = -53.00, 95% CI [-95.56, -10.05], $p = 0.02$), TNF-a MD = -53.05, 95%CI [-68.36, -37.73], $p < 0.00001$, and increased the serum levels of IL-10 (MD = 37.73, 95% CI [16.92, 58.54], $p = 0.0004$). Ulinastatin administration did not lead to any difference in the occurrence of adverse events.

Conclusions: Ulinastatin improved all-cause mortality and other related outcomes in patients with sepsis or septic shock. The results of this meta-analysis suggest that ulinastatin may be an effective treatment for sepsis and septic shock.

Keywords: sepsis, ulinastatin, mortality, inflammatory cytokine, immune system

INTRODUCTION

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al., 2016). It is the major cause of death in intensive care units (Martin et al., 2003). Epidemiological studies in the United States have shown that 750,000 cases are diagnosed with severe sepsis annually, and 215,000 deaths occur every year (Angus et al., 2001). Owing to advances in the management of sepsis, such as early fluid resuscitation, early administration of antibiotics, and advances in supportive care, such lung-protective mechanical ventilation, the risk of sepsis-associated death has been decreasing. However, the mortality of sepsis remains high (Stoller et al., 2016).

The mechanism of sepsis is complicated (Armstrong et al., 2017; Minasyan, 2017). Sepsis initiates a complex interplay of host pro-inflammatory and anti-inflammatory processes (Hotchkiss et al., 2013; Chousterman et al., 2017). Simultaneously, both inflammatory response and immunosuppression are involved in sepsis (Hotchkiss et al., 2013; Hotchkiss and Crouser, 2015). Serum concentrations of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), and many other cytokines or chemokines are increased after the onset of sepsis (Chousterman et al., 2017; Rajaee et al., 2018). Immunoparalysis caused by the apoptosis of many immune cells, including T cells, B cells, dendritic cells, and neutrophils, is another dominant problem in sepsis patients; this state in turn results in a depressed immune system and failure in the elimination of pathogens and maintenance of immune balance (Hotchkiss et al., 2013; Girardot et al., 2017). Furthermore, anti-apoptosis therapy by blocking receptors or inhibitors of apoptotic pathway can reduce mortality in sepsis models (Zhang et al., 2010; Harjai et al., 2013). Blocking of programmed cell death receptor-1 (PD-1) also demonstrated a potential toward the reduction of sepsis-associated mortality (Zhang et al., 2010; Hotchkiss et al., 2013; Patera et al., 2016).

It is known that serine proteases are involved in systemic inflammation and cell apoptosis (Wong, 1998; Wiedow and Meyer-Hoffert, 2005). Urinary trypsin inhibitor (also called ulinastatin or UTI) is an important protease inhibitor found in human urine, blood, and other tissues (Linder and Russell, 2014). It has been shown that UTI plays an anti-inflammatory role by decreasing the phosphorylation of p38 mitogen-activated protein kinase (p38-MAPK) and nuclear factor-κB (NF-κB) activation as well as an anti-apoptotic role by protecting the mitochondria and scavenging oxygen free radicals (Shu et al., 2014; Li et al., 2016). The study of UTI mechanism revealed that UTI can decrease the level of inflammatory mediators and reduce the frequency of immune cell apoptosis in sepsis models. Therefore, UTI has been proposed as a potentially new therapeutic option for the treatment of sepsis and multiple organ dysfunction syndrome (MODS) (Linder and Russell, 2014; Atal and Atal, 2016).

Recently, clinical trials in sepsis patients treated with UTI or UTI combined with thymosin α1 showed a survival benefit trend (Wu et al., 2013a; Karnad et al., 2014). Meanwhile, five meta-analysis of studies in sepsis patients either UTI administration alone or a combination of UTI and thymosin α1 have been published (Han et al., 2015; Li et al., 2015; Feng et al., 2016; Wang et al., 2016; Liu et al., 2017). Among these five meta-analysis, two meta-analysis (Feng et al., 2016; Liu et al., 2017) analyzed the effect of using UTI alone and no significant difference between UTI group and control group in the 28-day mortality. Given that the results of these two meta-analysis are based on subgroup analysis, which included the same two trials (Wu et al., 2013a; Karnad et al., 2014), it is difficult to prove the effect of UTI alone. At present, it remains unclear whether the beneficial impact is rendered by UTI, thymosin α1, or the combination. Therefore, we pooled the randomized controlled trials that involved the use of UTI alone in order to clarify the efficacy of UTI in sepsis.

MATERIALS AND METHODS

In accordance to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement for reporting systematic reviews and meta-analysis (Liberati et al., 2009), two groups of the present authors [(HW and LY) and (YT and BH)] independently conducted literature searches, established the study inclusion and exclusion criteria, performed quality assessment, and extracted data. If a consensus was not reached, it was resolved by the senior authors (ZL and PC). The flow graph is shown in **Figure 1**.

Study Registration

Registration number in PROSPERO: CRD42018110751, an international prospective register of systematic reviews.

Search Strategy

We searched for randomized controlled trials in sepsis published on or before September 01, 2018, regardless of language, publication type, or study region. The electronic database includes PubMed, Medline, Embase, and China National Knowledge Infrastructure (CNKI). The key words that were searched and their combinations in (title/abstract) are shown in **Table 1**.

Selection Criteria

Studies were included if they met the following criteria: 1) participants: patients who were diagnosed with sepsis, severe sepsis, or septic shock; 2) type of interventions: use of UTI alone regardless of treatment duration; 3) research design: either randomized controlled trial (RCT) or prospective cohort study. Review articles, animal experimental studies, case reports, and letters that did not describe outcomes or were not published as full reports were excluded. In addition, we only included the most updated and completed studies in case of duplicated publication.

Outcomes and Data Extraction

The primary outcome was all-cause mortality. The secondary outcomes were changes in the serum levels of IL-6, IL-10, and TNF-α, the incidence rate of MODS, and changes in Acute Physiology, Age, Chronic Health Evaluation II (APACHE II) scores. We also collected the following information: study

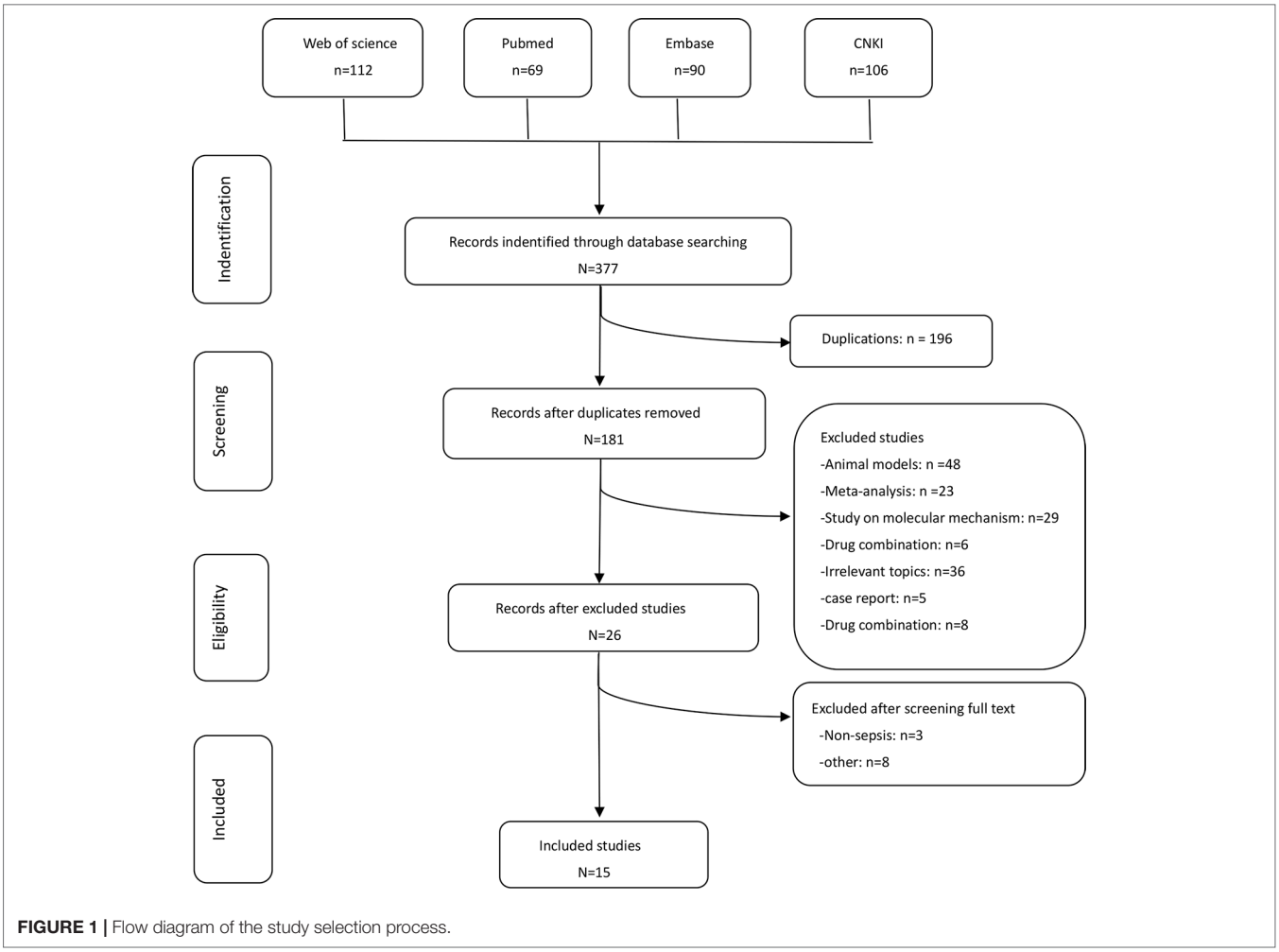


FIGURE 1 | Flow diagram of the study selection process.

TABLE 1 | Search terms and phrases used in the meta-analysis.

#1 ulinastatin
#2 UTI
#3 urinary protease inhibitor
#4 sepsis
#5 sept*mia
#6 effect*
#7 treatment
#8 therap*
#9 Systemic Inflammatory Response Syndrome
#10 SIRS
#11 MODS
#12 Multiple organ dysfunction syndrome
#11 #1or #2 or #3
#12 #4 or #5 or #9 or #10 or #11 or #12
#13 #11 and #12 and #6
#14 #11 and #12 and #7

The means of "" is truncation.*

design, year of study, country, study period, the number of patients included, intervention methods, and adverse events. The main characteristics of the included studies are shown in **Table 2**. For the continuous variables, we acquired data according to the following method. For calculating the mean in this meta-analysis, we employed the formula $X = X_2 - X_1$, where X represents the mean applied in this meta-analysis, X1 represents the baseline mean, and X2 represents the endpoint mean. For calculating the standard deviation (SD) in this metaanalysis, we chose to employ the formula $S^2 = S_1^2 + S_2^2 - 2 \times R \times S_1 \times S_2$, where S represents the standard deviation applied in this meta-analysis, S1 represents the baseline SD, and S_2 represents the endpoint SD. R = 0.5 in the meta-analysis, which was described in the Cochrane Handbook. All data were independently extracted by two authors (HW and LY). HW entered data into the computer and LY checked them.

Quality Assessment

First, two reviewers independently assessed the eligibility of articles identified during the initial search strategy. Then, the quality of all included studies was evaluated according to the modified Jadad scale, which can intuitively assess the quality of the included RCTs (Jadad et al., 1996). The studies were rated as low quality and high quality under scores of 1–3 and 4–7, respectively. In this meta-analysis, three studies were regarded as low quality and 12 studies were regarded as high quality. Moreover, detailed scoring results are shown in **Table 3**.

TABLE 2 | The characteristics of the included studies.

Author (year published)	Country	Study period	Study Type	Total No. of patients	Number of patients		Mean Age, yrs		Diagnosis	Interventions		Outcomes		Adverse effects	Quality score
					UTI group	Control group	UTI group	Control group		UTI group	Control group	Primary outcome	Secondary outcomes		
Fang and Zhao, (2017)	China	2013.03-2015.05	RCT	96	49	47	56.7 ± 12.5	59.3 ± 11.6	severe sepsis	30,0000 IU q8h×5d	Antibiotics standard care	28-day all-cause mortality	PCT, CRP, IL-6, TNF-a	none	☆☆☆☆☆☆
Choudhuri et al. (2015)	India	2012.10-2014.05	RCT	104	68	36	P > 0.05		sepsis	NR	NR	28-day all-cause mortality	VDs, length of ICU stay, VASDs, occurrence of MODS	none	☆☆☆
Karnad et al. (2014)	India	2009.09-2010.06	RCT	114	55	59	37.5 ± 12.9	36.7 ± 12.5	sepsis	20,0000 IU q12h×5d	equivalent normal saline	28-day all-cause mortality	VDs VFDs hospital stay, APACHE II score	none	☆☆☆☆☆☆☆
Wu et al. (2013b)	China	2011.10-2012.10	RCT	60	30	30	54.3 ± 16.2		sepsis	30,0000 IU q8h×5d	equivalent normal saline	28-day all-cause mortality	MODS,IL-10,IL-6 CD4,CD25,IL-17,HLA-DR	none	☆☆☆☆☆
Sung et al. (2009)	Korea	2005.01-2008.06	PC	169	43	126	61 ± 18	61 ± 17	severe sepsis septic shock	100,0000 IU qd	Antibiotics standard care	mortality	SOFA score	none	☆☆☆
Shao et al. (2005)	China	NR	RCT	60	30	30	43.3 ± 9.2		sepsis	10,0000 IU q8h×5d	Antibiotics standard care	mortality	IL-6, IL-10, TNF-a, CRP	none	☆☆☆☆☆☆
Pavan Kumar et al. (2017)	India	2014.10-2017.10	PO	225	87	138	P > 0.05		sepsis	20,0000 IU q12h×5d	Antibiotics standard care	all-cause mortality	VFDs VASFDs	none	☆☆☆
Chen et al. (2015)	China	2013.07-2014.06	RCT	50	25	25	43.6 ± 5.8	41.7 ± 3.8	severe sepsis	20,0000 IU q12h×7 d	equivalent normal saline	28-day all-cause mortality	IL-8,TNF-α,IL-6,IL-10	none	☆☆☆☆
Tang et al. (2013)	China	NR	RCT	74	37	37	31-52		severe sepsis	20,0000 IU q12h×7d	equivalent normal saline	all-cause mortality	IL-8, TNF-α,IL-6,IL-10	none	☆☆☆☆
Jiang et al. (2006)	China	2001.12-2005.12	RCT	78	39	39	56 ± 21	54 ± 16	Severe sepsis septic shock	20,0000 IU qd×3d	equivalent normal saline	NR	IL-8,IL-1, TNF-α,IL-6	none	☆☆☆☆
Ni et al. (2008)	China	2006.1-2007.2	RCT	42	21	21	60.18 ± 19.08	59.39 ± 21.11	severe sepsis	10000 IU/kg/d q12h×5d	equivalent normal saline	28-day mortality	IL-10, TNF-α, APACHE II score	none	☆☆☆☆☆
Fang et al. (2005)	China	2003.09-2004.02	RCT	56	28	28	57 ± 16	61 ± 16	sepsis	20,0000 IU q12h×5d	equivalent normal saline	28-day mortality	IL-8, TNF-α,IL-6, APACHE II score	rash	☆☆☆☆
Dai et al. (2016)	China	2013.07-2014.06	RCT	86	43	43	59.45 ± 6.54	59.32 ± 6.15	severe sepsis	20,0000 IU q12h×5d	equivalent normal saline	NR	IL-8, TNF-α,IL-10, APACHE II score	nausea, fatigue and rash	☆☆☆☆☆
Wang et al. (2007)	China	2004.1-2006.12	RCT	84	44	40	55.3 ± 24.5	52.1 ± 16.3	sepsis	20,0000 IU q12h×7d	equivalent normal saline	NR	TNF-α,IL-6,IL-10,IL-8,IL-1	none	☆☆☆☆
Wu et al. (2016)	China	2011-2012	RCT	60	31	29	48.71 ± 30.15	50.09 ± 29.11	sepsis	20,0000 IU q8h×8d	equivalent normal saline	28-day mortality	TNF-α,IL-10 APACHE II score	None	☆☆☆☆

RCT, Randomized controlled study; PC, prospective case–control study, PO, prospective observational study; p > 0.05, no difference in the baseline of mean age; VFDs, ventilator-free days; VDs, ventilator days; VASFDs, vasopressor-free days; VASDs, vasopressor days; NR, It was not given in the original article.

TABLE 3 | The modified Jadad questionnaire for the included studies.

Study, year (Station)	Random sequence production			Allocation concealment			Blinding metod			Withdrawals and dropouts		Score
	Adequate 2'	Unclear 1'	Inadequate 0'	Adequate 2'	Unclear 1'	Inadequate 0'	Unused 0'	Adequate 2'	Unclear 1'	Inadequate 0'	Description 1'	Undescribed 0'	
Wang et al., 2007		1			1			2				0	4
Jiang et al. (2006)		1			1			2				0	4
Karnad et al., 2014		2			2			2			1		7
Wu et al., 2013b		2			1			2				0	5
Sung et al., 2009		1			1				1			0	3
Wu et al., 2016		1			1			2				0	4
Dai et al. (2016)		2			1			2				0	5
Tang et al. (2013)		1			1			2				0	4
Chen et al., 2015		1			1			2				0	4
Fang et al. (2005)		1			1			2				0	4
Ni et al. (2008)		2			1			2				0	5
Shao et al., 2005	2			2				2				0	6
Fang and Zhao, 2017	2			2				2				0	6
Pavan Kumar et al., 2017		1			1				1			0	3
Choudhuri et al., 2015		1			1				1			0	3

1–3 score, low quality; 4–7 score, high quality.

Statistical Analysis

In this meta-analysis, all statistical calculations and analysis were performed using Review Manager 5.3 (Cochrane collaboration, Oxford, UK). According to the results of statistical analysis, we divided the type of data into dichotomous and continuous. For the dichotomous data, such as mortality, the incidence of MODS, and adverse events, we calculated the odds ratios (OR), 95% confidence intervals (CIs) of every included study, and the overall Mantel–Haenszel (M-H). For the continuous data, such as IL-6, IL-10, and TNF-α levels and the APACHE II score, we calculated the mean difference (MD) and 95% CIs. The statistical heterogeneity was examined using chi-square and I^2 statistical tests as well as P values. At first, we used a fixed-effects model, but then chose to employ the random-effects model if I^2 was ≥50%.

Because patients with sepsis have a high mortality rate and not all studies report a 28-day mortality rate, we chose all-cause mortality to be our primary outcome. Sensitivity analysis was used to judge whether the study results were statistically significant. For eliminating publication bias, we used the funnel plot method.

RESULTS

Description of Eligible Studies

We identified 15 (Fang and Chen, 2005; Jiang et al., 2006; Wang et al., 2007; Ni et al., 2008; Sung et al., 2009; Tang, 2013; Wu et al., 2013b; Karnad et al., 2014; Chen et al., 2015; Dai and Wang, 2016; Wu et al., 2016; Fang and Zhao, 2017; Choudhuri et al., 2015; Shao et al., 2005; Pavan Kumar et al., 2017) potential studies that included a total of 1358 patients: 630 patients in the UTI group and 728 patients in the control group. Thirteen RCTs and two prospective studies were included in this meta-analysis. The specific method for identifying studies and establishing the inclusion and exclusion criteria is shown in **Figure 1**. Eleven studies were published from China, three from India, and one from Korea.

Primary Outcomes

All-Cause Mortality

We extracted the data from 12 studies (Fang and Chen, 2005; Shao et al., 2005; Ni et al., 2008; Sung et al., 2009; Tang, 2013; Wu et al., 2013b; Karnad et al., 2014; Chen et al., 2015; Choudhuri et al., 2015; Wu et al., 2016; Fang and Zhao, 2017; Pavan Kumar Rao et al., 2017) and 1110 participants were classified into two groups to assess all-cause mortality. All-cause mortality was significantly lower in the UTI group than in the control group (OR = 0.48, 95% CI [0.35, 0.66], $p < 0.00001$), and heterogeneity was low ($x^2 = 12.57$, $p = 0.32$, $I^2 = 13\%$). The results are shown in **Figure 2**.

Secondary Outcomes

Levels of IL-6

We obtained the related data from eight studies (Fang and Chen, 2005; Shao et al., 2005; Jiang et al., 2006; Wang et al., 2007; Tang, 2013; Wu et al., 2013b; Chen et al., 2015; Fang and Zhao, 2017) (558 participants in two groups) to analyze the serum levels of IL-6. The serum level of IL-6 at the time of hospital admission was not different between the UTI and control groups. After

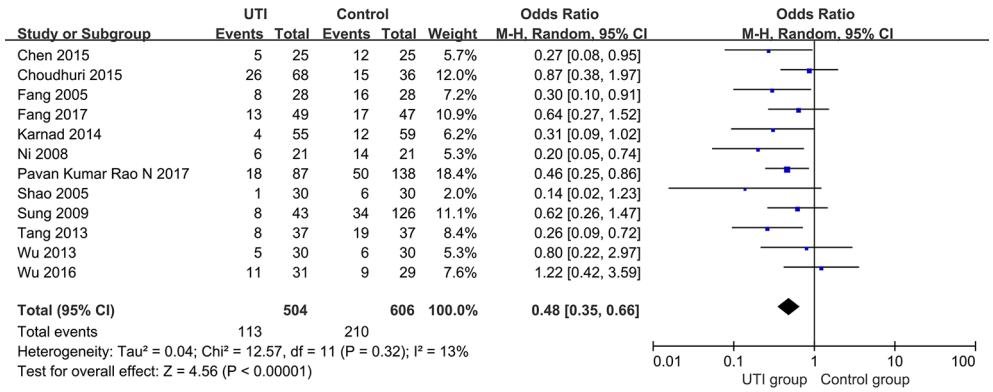


FIGURE 2 | All-cause mortality of the included studies.

treatment, IL-6 was significantly less in the UTI group than in the control group (MD = -53.00, 95% CI [-95.56,-10.05], p = 0.02), and a obvious heterogeneity in the results was observed (x^2 = 410.24, p < 0.00001, I^2 = 98%). The results are shown in **Figure 3A**.

Levels of TNF-α

We collected the related data from 10 studies (Fang and Chen, 2005; Shao et al., 2005; Jiang et al., 2006; Wang et al., 2007; Ni et al., 2008; Tang, 2013; Chen et al., 2015; Dai and Wang, 2016; Wu et al., 2016; Fang and Zhao, 2017) (686 participants in two groups)

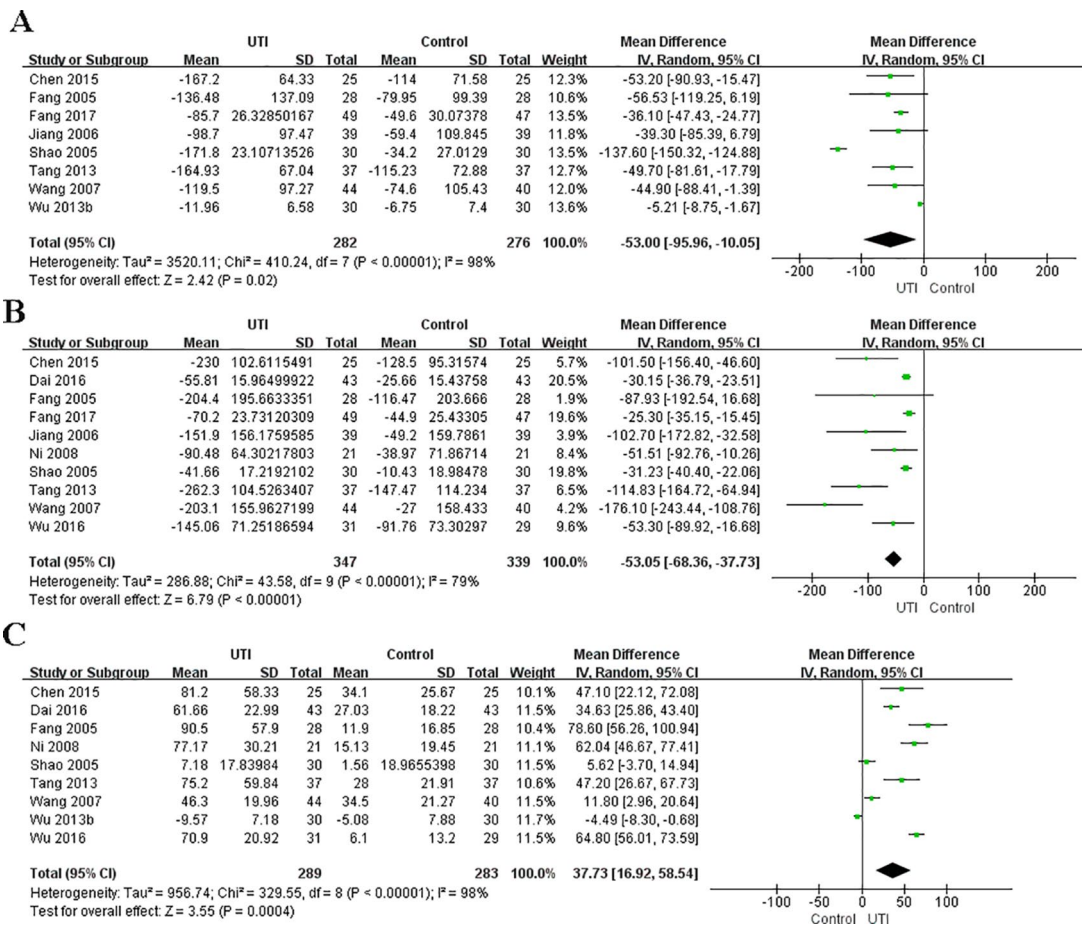


FIGURE 3 | Ulinastatin regulates the levels of pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory factor: IL-6 **(A)**, TNF-α **(B)**. Anti-inflammatory factors: IL-10 **(C)**.

to analyze the serum levels of TNF-α. The level of TNF-α at the time of hospital admission was not different between the UTI and control groups. After treatment, TNF-α was significantly less in the UTI group than in the control group (MD = -53.05, 95%CI [-68.36,-37.73], $p < 0.00001$), and an obvious heterogeneity was observed in the results ($x^2 = 43.58$, $p < 0.00001$, $I^2 = 79\%$). The results are shown in **Figure 3B**.

Levels of IL-10

We gained the related data from nine studies (Fang and Chen, 2005; Shao et al., 2005; Wang et al., 2007; Ni et al., 2008; Tang, 2013; Wu et al., 2013b; Chen et al., 2015; Dai and Wang, 2016; Wu et al., 2016) (572 participants in two groups) to analyze the serum levels of IL-10. The serum level of IL-10 at the time of hospital admission was not different between the UTI and control groups. After treatment, IL-10 was significantly greater in the UTI group than in the control group (MD = 37.73, 95% CI [16.92, 58.54], $p = 0.0004$), and an obvious heterogeneity was observed in the results ($x^2 = 329.55$, $p < 0.0001$, $I^2 = 98\%$). The results are shown in **Figure 3C**.

The Apache II Score

The APACHE II score at the time of hospital admission were not different between the UTI and control groups. We extracted the data from four studies (Ni et al., 2008; Fang et al., 2005; Dai and Wang, 2016; Wu et al., 2016) (244 participants in two groups) to assess this change. After treatment, the APACHE II scores were significantly less in the UTI group than in the control group MD = -3.18, 95%CI [-4.01, -2.35], $p < 0.00001$), and heterogeneity was low ($x^2 = 4.51$, $p = 0.21$, $I^2 = 33\%$). The results are shown in **Figure 4**.

The Incidence of MODS

We extracted the data from three studies (Shao et al., 2005; Karnad et al., 2014; Pavan Kumar Rao et al., 2017) (399 participants in included in two groups) to assess the incidence of MODS. After treatment, the incidence of MODS was significantly less in the UTI groups than in the control groups (OR = 0.3, 95% CI [0.18–0.49], $p < 0.00001$), and heterogeneity was not observed in the results ($x^2 = 0.58$, $p = 0.75$, $I^2 = 0\%$). The result is shown in **Figure 5**.

Publication Bias and Sensitivity Analysis

All included studies involved the use of UTI for treating sepsis patients. Using all-cause mortality as the main variable, the included studies were evaluated for the effect of study size. The funnel plot demonstrated a balanced and or a symmetrical shape, suggesting no significant publication bias. In addition, Egger's test also demonstrated a statistically significant symmetry ($p = 0.183$). Therefore, the potential publication bias had no significant influence on the results (**Figure 6**).

Sensitivity analysis was conducted by the leave-one-out method and checking the consistency of the overall effect estimate. For IL-6, we found that the I^2 value decreased to 0% after excluding the studies conducted by Shao et al. (2005) and Wu et al. (2013b). For TNF-α, we found that the I^2 value decreased to 54% after excluding the studies conducted by Tang et al. (2013) and Wang et al. (2007). For IL-10, we found that the I^2 value decreased to 84% after removing the study by Shao et al. (2005), Wang et al. (2007) and Wu et al. (2013b). We believe that the high heterogeneity may arise from factors such as sample size, different measuring instruments, and design methods.

DISCUSSION

UTI is a multifunctional Kunitz-type serine protease inhibitor found in human urine and blood. UTI is a member of inter-a-inhibitor (IaI) family, which is produced by hepatocytes (Linder and Russell, 2014). It was originally used to treat acute pancreatitis or hyperthermia (Itaba et al., 2013;

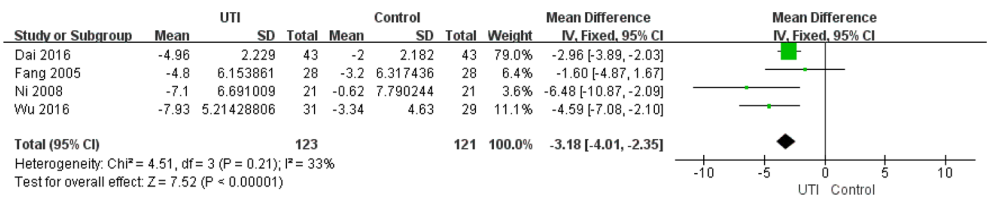


FIGURE 4 | Ulinastatin reduces the APACHE II score of sepsis patients.

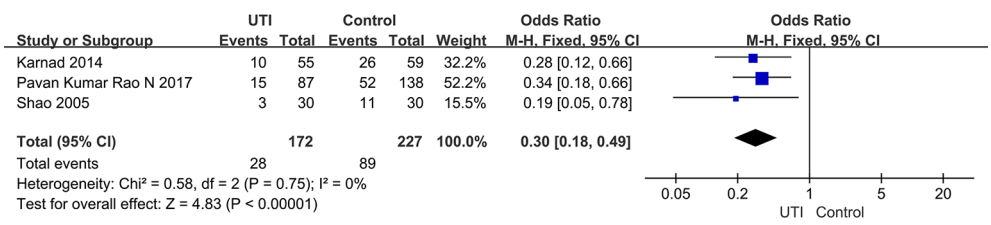
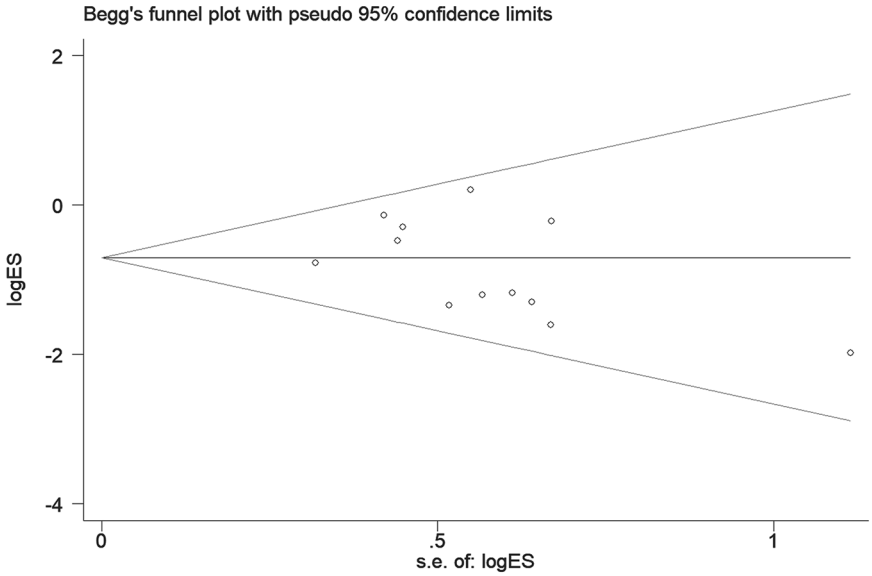


FIGURE 5 | Ulinastatin reduces the incidence of multiple organ dysfunction syndrome (MODS).



Egger's test

Std_Eff	Coef.	Std. Err.	t	P>t	[95% Conf.	Interval]
slope	.0517594	.5522227	0.09	0.927	-1.178669	1.282188
bias	-1.546393	1.083454	-1.43	0.184	-3.960479	.8676934

FIGURE 6 | The Begg's test and Egger's test for all-cause-mortality. Begg's test: rank correlation test; Egger's test: linear regression method; ES, effect size; 95% CI, 95% confidence interval.

Zhang et al., 2016). Subsequently, more studies revealed its use in anti-inflammation and the protection of liver function as well as in cardiopulmonary bypass and lung disease treatment (Song et al., 2011; Li et al., 2016). In such conditions, UTI can inhibit the inflammatory response, scavenge oxygen free radicals, and shorten the time of tracheal intubation and ventilation (Yang et al., 2011; Hui et al., 2014). At present, UTI has been evidenced to provide an attractive "rescue" therapeutic option for endotoxin-related inflammatory disorders such as disseminated intravascular coagulation (DIC), acute lung injury, and acute liver injury (Inoue and Takano, 2010). Recently, UTI has been demonstrated to play a vital role in sepsis. It is well known that the immune state of sepsis patients undergoes complex changes from the onset of hyper-inflammatory response in the early phase to the immune paralysis in the late phase. To date, no drug has been specifically approved to treat sepsis in human. Recent studies show that UTI has the capacity to reduce inflammation and protect cells and has a potential survival benefit in sepsis and MODS (Linder and Russell, 2014; Atal and Atal, 2016; Chang et al., 2017a). In the past, the research on UTI treatment for sepsis has mainly been conducted in China, and the results suggested UTI administration can reduce sepsis patient mortality. In 2014, a randomized, controlled, double-blind, and multi-center trial was conducted in India, which revealed the survival benefit of UTI in patients with sepsis (Karnad et al., 2014). A meta-analysis of the effects of UTI combined with thymosin α1 revealed a reduction in mortality. To better clarify the efficacy of UTI or thymosin α1 administration on sepsis patients, we pooled the RCTs involving treatment with UTI alone. In this meta-analysis, 13 relevant RCTs from three countries and two prospective studies were included. The results showed that in patients with sepsis or septic shock UTI was associated with a significant decrease in all-cause mortality and improvements in both inflammatory cytokine profiles and APACHE II scores. Mortality is the most important index for efficacy evaluation. Studies conducted in sepsis models support that UTI is capable of reducing sepsis-related mortality. The survival benefits were also observed in most clinical trials conducted in different countries. Several trials failed to achieve positive results, probably owing to an insufficient sample size and differences in patients, trials design, and other clinical factors.

Anti-inflammation is one of the most important properties of UTI. It is well known that systemic inflammatory response plays a key role in organ damage or death in sepsis. Agents directed at a single inflammatory mediator have not been shown to have a protective effect in sepsis patients. These results suggest that a single anti-inflammatory agent cannot disrupt the complicated inflammatory network. However, the removal of blood mediators by continuous renal replacement therapy (CRRT) facilitated the achievement of a survival benefit in patients with sepsis or septic shock (Liu et al., 2011; Servillo et al., 2013). Similarly with CRRT, UTI demonstrated a capacity to decrease diverse inflammatory mediator factors such as IL-1, IL-8, IL-6, HMGB1, and other mediators. UTI also inhibits inflammation by suppressing the infiltration of neutrophils and release of elastase and inflammatory mediators from neutrophils. UTI can also suppress MAPK-signaling pathway, which mediates the release of inflammatory

cytokines such as TNF-α, IL-1, and IL-6 (Inoue and Takano, 2010; Fang et al., 2018). Recently, a retrospective study of 263 critically ill patients with sepsis found that 28-day mortality decreased significantly with UTI (Xu et al., 2018). The authors concluded that 35% of the total effect of UTI was associated with the reduction in C-reactive protein (CRP), a major marker of inflammation.

Anti-apoptosis is another property of UTI. It has been observed that UTI can reduce apoptosis of endothelial cells, lymphocytes, intestinal epithelium, neurons, and renal cells during different diseases and in animal models (Li et al., 2014). It is well known that there are many immune cells, including lymphocytes, monocytes, and dendritic cells that undergo apoptosis during sepsis. It has been confirmed that UTI can protect cells from apoptosis though anti-oxidation and reduction of mitochondrial damage. It is known that apoptosis contributes to immunoparalysis and death of sepsis patients (Hotchkiss and Nicholson, 2006; Hotchkiss et al., 2013; Chang et al., 2017b). Anti-apoptosis in sepsis models, *via* increase in Bcl-2 expression or blocking of CD95, reduced the incidence of sepsis-related mortality (Hotchkiss and Nicholson, 2006; Zhang et al., 2010; Sun et al., 2011; Liu et al., 2013). In clinical trials, anti-immune cell apoptosis with anti-PD-1 or anti-PD-L1 also showed potential in sepsis treatment (Zhang et al., 2010; Patera et al., 2016). These studies suggest that cell protection may also be involved in UTI-related survival benefit in patients with sepsis.

LIMITATIONS

Although this meta-analysis reveals the potential benefits of UTI inpatients with sepsis, these trials were conducted mainly in single centers and the sample sizes were small. Recently, a retrospective observational study conducted in a single intensive care unit (ICU) by Uchida et al. (Uchida et al., 2018) found that UTI was not associated with a mortality benefit in elderly patients with established multiple organ failure from a variety of causes, only a minority of which were sepsis related. However, UTI use was associated with reduced time on both mechanical ventilators and vasoactive drugs. Thus, multicenter, large sample, randomized clinical trials are still urgently needed to further evaluate the effects of UTI in patients with sepsis. At present, ADJunctive Ulinastatin in Sepsis Treatment in China (ADJUST study), a large sample, multi-center, double-blind, randomized, parallel-group, placebo-controlled trial is being conducted in mainland China (Jiang et al., 2018). The aim of this trial is to further evaluate the efficacy and safety profiles of UTI.

CONCLUSIONS

UTI is associated with reductions in both all-cause mortality and the incidence of MODS, and improvements in both APACHE II scores and inflammatory cytokine profiles in patients with sepsis, severe sepsis, or septic shock. Large high quality RCTs are needed to confirm these promising results of UTI in sepsis and septic shock.

DATA AVAILABILITY STATEMENT

The data analyzed in this study was obtained from PubMed, Medline, Embase, and China National Knowledge Infrastructure (CNKI), the following licenses apply. Requests to access these datasets should be directed to HW, 970092671@qq.com.

AUTHOR CONTRIBUTIONS

ZL conceived and designed the study. HW, LY, YT, BH, ZL, and PC conducted the literature search, read initial abstracts, extracted data from potential eligible studies, and conducted the statistical analyses. HW and BL wrote the first draft of the manuscript. ZL, PC, RL, and BL contributed with manuscript writing, concrete suggestions, and manuscript revision.

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REFERENCES

Angus, D. C., Linde-Zwirble, W. T., Lidicker, J., Clermont, G., Carcillo, J., and Pinsky, M. R. (2001). Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit. Care Med.* 29 (7), 1303–1310. doi: 10.1097/00003246-200107000-00002

Armstrong, B. A., Betzold, R. D., and May, A. K. (2017). Sepsis and septic shock strategies. *Surg. Clin. North Am.* 97 (6), 1339–1379. doi: 10.1016/j.suc.2017.07.003

Atal, S. S., and Atal, S. (2016). Ulinastatin - a newer potential therapeutic option for multiple organ dysfunction syndrome. *J. Basic Clin. Physiol. Pharmacol.* 27 (2), 91–99. doi: 10.1515/jbcpp-2015-0003

Chang, P., Liu, J., Yu, Y., Cui, S. Y., Guo, Z. H., and Chen, G. M. (2017a). Alpha-lipoic acid suppresses extracellular histone-induced release of the infammatory mediator tumor necrosis factor-alpha by macrophages. *Cell Physiol. Biochem.* 42 (6), 2559–2568. doi: 10.1159/000480217

Chang, P., Mo, B., Cauvi, D. M., Yu, Y., Guo, Z., and Zhou, J. (2017b). Grape seed proanthocyanidin extract protects lymphocytes against histone-induced apoptosis. *PeerJ* 5, e3108. doi: 10.7717/peerj.3108

Chen, W., Peng, S., and Chen, Y. (2015). Comparative observation of curative effects by ulinastatin and conventional method in the treatment of severe sepsis. *Chinese J. Modern Drug App.* 9 (7), 1–2, 3. doi: 10.14164/j.cnki.cn11-5581/r.2015.07.001

Choudhuri, A. H., Tyagi, R., Tyagi, R., Agarwal, D., and Uppal, R. (2015). Early use of ulinastatin reduces Multiorgan Dysfunction (MODS) in septic shock following anastomotic failure. *Clin. Therapeutics* 37 (8), e112.

Chousterman, B. G., Swirski, F. K., and Weber, G. F. (2017). Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol.* 39 (5), 517–528. doi: 10.1007/s00281-017-0639-8

Dai, Q. X., and Wang, C. H. (2016). Effects of urinary trypsin inhibitor on serum levels of TNF-a, IL-8 and IL-10 in patients with severe sepsis. *Chinese J. Biochem. Pharmaceutics* 36 (3), 146–148. doi: 10.3969/j.issn.1005-1678.2016.03.48

Fang, Q., and Chen, P. (2005). A Study on clinical efficacy of ulinastatin in severe sepsis patients and its mechanism of action. *Chinese J. Of Infect. And Chemother.* 5 (1), 13–16. doi: 10.3321/j.issn:1009-7708.2005.01.003

Fang, Q., and Zhao, X. (2017). Clinical effect of combined ulinastatin and continuous renal replacement therapy on management of severe sepsis with

acute kidney injury. *Tropical J. Pharmaceutical Res.* 16 (4), 925–930. doi: 10.4314/tjpr.v16i4.26

Fang, M., Zhong, W.-H., Song, W.-L., Deng, Y.-Y., Yang, D.-M., and Xiong, B. (2018). Ulinastatin ameliorates pulmonary capillary endothelial permeability induced by sepsis through protection of tight junctions via inhibition of TNF-alpha and related pathways. *Front. Pharmacol.* 9, 823. doi: 10.3389/fphar.2018.00823

Feng, Z., Shi, Q., Fan, Y., Wang, Q., and Yin, W. (2016). Ulinastatin and/or thymosin alpha1 for severe sepsis: a systematic review and meta-analysis. *J. Trauma Acute Care Surg.* 80 (2), 335–340. doi: 10.1097/ta.0000000000000909

Girardot, T., Rimmele, T., Venet, F., and Monneret, G. (2017). Apoptosis-induced lymphopenia in sepsis and other severe injuries. *Apoptosis* 22 (2), 295–305. doi: 10.1007/s10495-016-1325-3

Han, D., Shang, W., Wang, G., Sun, L., Zhang, Y., and Wen, H. (2015). Ulinastatin- and thymosin alpha1-based immunomodulatory strategy for sepsis: a meta-analysis. *Int. Immunopharmacol.* 29 (2), 377–382. doi: 10.1016/j.intimp.2015.10.026

Harjai, M., Bogra, J., Kohli, M., and Pant, A. B. (2013). Is suppression of apoptosis a new therapeutic target in sepsis?. *Anaesth. Intensive Care* 41 (2), 175–183. doi: 10.1177/0310057X1304100207

Hotchkiss, R. S., and Crouser, E. (2015). Imaging apoptosis in sepsis–A technology we would die for!. *Crit. Care Med.* 43 (11), 2506–2508. doi: 10.1097/ccm.0000000000001289

Hotchkiss, R. S., and Nicholson, D. W. (2006). Apoptosis and caspases regulate death and inflammation in sepsis. *Nat. Rev. Immunol.* 6 (11), 813–822. doi: 10.1038/nri1943

Hotchkiss, R. S., Monneret, G., and Payen, D. (2013). Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat. Rev. Immunol.* 13 (12), 862–874. doi: 10.1038/nri3552

Hui, L., Shen, F., Chang, H., Li, X., Gao, G., and Ma, J. (2014). Effects of ulinastatin on cerebral oxygen metabolism and CRP levels in patients with severe traumatic brain injury. *Exp. Therapeutic Med.* 7 (6), 1683–1686. doi: 10.3892/etm.2014.1666

Inoue, K.-I., and Takano, H. (2010). Urinary trypsin inhibitor as a therapeutic option for endotoxin-related inflammatory disorders. *Expert Opin. Investig. Drugs* 19 (4), 513–520. doi: 10.1517/13543781003649533

Itaba, S., Nakamura, K., Aso, A., Tokunaga, S., Akiho, H., and Ihara, E. (2013). Prospective, randomized, double-blind, placebo-controlled trial of ulinastatin for prevention of hyperenzymemia after double balloon endoscopy via the antegrade approach. *Dig. Endosc.* 25 (4), 421–427. doi: 10.1111/den.12014

Jadad, A. R., Moore, R. A., Carroll, D., Jenkinson, C., Reynolds, D. J., and Gavaghan, D. J. (1996). Assessing the quality of reports of randomized clinical trials: is blinding necessary?. *Control Clin. Trials* 17 (1), 1–12. doi: 10.1016/0197-2456(95)00134-4

Jiang, L. Y., Hong,Y. L., and Xing, C. J. (2006). The effect of Ulinastatin on the delivery of cytokines in patients with septic shock. *Chinese J. Of Emergency Med.* 15 (1671-0282), 1136–1138. doi: 10.3760/j.issn:1671-0282.2006.12.020

Jiang, W., Yu, X., Sun, T., Chai, Y., Chang, P., and Chen, Z. (2018). ADJunctive Ulinastatin in Sepsis Treatment in China (ADJUST study): study protocol for a randomized controlled trial. *Trials* 19 (1), 133. doi: 10.1186/s13063-018-2513-y

Karnad, D. R., Bhadade, R., Verma, P. K., Moulick, N. D., Daga, M. K., and Chafekar, N. D. (2014). Intravenous administration of ulinastatin (human urinary trypsin inhibitor) in severe sepsis: a multicenter randomized controlled study. *Intensive Care Med.* 40 (6), 830–838. doi: 10.1007/s00134-014-3278-8

Li, G., Li, T., Li, Y., Cai, S., Zhang, Z., and Zeng, Z. (2014). Ulinastatin inhibits oxidant-induced endothelial hyperpermeability and apoptotic signaling. *Int. J. Clin. Exp. Pathol.* 7 (11), 7342–7350.

Li, C., Bo, L., Liu, Q., and Jin, F. (2015). Thymosin alpha1 based immunomodulatory therapy for sepsis: a systematic review and meta-analysis. *Int. J. Infect. Dis.* 33, 90–96. doi: 10.1016/j.ijid.2014.12.032

Li, C., Ma, D., Chen, M., Zhang, L., Zhang, L., and Zhang, J. (2016). Ulinastatin attenuates LPS-induced human endothelial cells oxidative damage through suppressing JNK/c-Jun signaling pathway. *Biochem. Biophys. Res. Commun.* 474 (3), 572–578. doi: 10.1016/j.bbrc.2016.04.104

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gotzsche, P. C., and Ioannidis, J. P. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 339, b2700. doi: 10.1136/bmj.b2700

Linder, A., and Russell, J. A. (2014). An exciting candidate therapy for sepsis: ulinastatin, a urinary protease inhibitor. *Intensive Care Med.* 40 (8), 1164–1167. doi: 10.1007/s00134-014-3366-9

Liu, Z.-G., Zhang, J., He, Y., Chang, P., Gu, D.-S., and Luo, Y.-W. (2011). Impact of transfusion of apoptotic and necrotic thymocytes on the survival of mice with sepsis. *J. Of Southern Med. Univ.* 31 (6), 960–964.

Liu, Z. G., Ni, S. Y., Chen, G. M., Cai, J., Guo, Z. H., and Chang, P. (2013). Histones-mediated lymphocyte apoptosis during sepsis is dependent on p38 phosphorylation and mitochondrial permeability transition. *PLoS One* 8 (10), e77131. doi: 10.1371/journal.pone.0077131

Liu, D., Yu, Z., Yin, J., Chen, Y., Zhang, H., and Fan, X. (2017). Effect of ulinastatin combined with thymosin alpha1 on sepsis: a systematic review and meta-analysis of Chinese and Indian patients. *J. Crit. Care* 39, 285–287. doi: 10.1016/j.jcrc.2017.02.005

Martin, G. S., Mannino, D. M., Eaton, S., and Moss, M. (2003). The epidemiology of sepsis in the United States from 1979 through 2000. *N. Engl. J. Med.* 348 (16), 1546–1554. doi: 10.1056/NEJMoa022139

Minasyan, H. (2017). Sepsis and septic shock: Pathogenesis and treatment perspectives. *J. Crit. Care* 40, 229–242. doi: 10.1016/j.jcrc.2017.04.015

Ni, H. Y., Fang, Q., and Zhang., Y. S. (2008). Effects of ulinastatin on Inflammatory response and curative effect in severe sepsis patients. *Chinese J. Of Crit. Care Med.* 28 (4), 342–344. doi: 10.3969/j.issn.1002-1949.2008.04.017

Patera, A. C., Drewry, A. M., Chang, K., Beiter, E. R., Osborne, D., and Hotchkiss, R. S. (2016). Frontline Science: defects in immune function in patients with sepsis are associated with PD-1 or PD-L1 expression and can be restored by antibodies targeting PD-1 or PD-L1. *J. Leukoc. Biol.* 100 (6), 1239–1254. doi: 10.1189/jlb.4HI0616-255R

Pavan Kumar Rao, N., Sv, S., and Nayak, K. S. (2017). Use of ulinastatin in renal failure patients developing sepsis. *Indian J. Nephrol.* 27, S68–S69.

Rajaee, A., Barnett, R., and Cheadle, W. G. (2018). Pathogen- and danger-associated molecular patterns and the cytokine response in sepsis. *Surg. Infect. (Larchmt)* 19 (2), 107–116. doi: 10.1089/sur.2017.264

Servillo, G., Vargas, M., Pastore, A., Procino, A., Iannuzzi, M., and Capuano, A. (2013). Immunomodulatory effect of continuous venovenous hemofiltration during sepsis: preliminary data. *Biomed. Res. Int.* 2013, 108951. doi: 10.1155/2013/108951

Shao, Y. M., Zhang, L. Q., Deng, L. H., and Yao, H. G. (2005). [Clinical study on effects of ulinastatin on patients with systemic inflammatory response syndrome]. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 17 (4), 228–230.

Shu, H., Liu, K., He, Q., Zhong, F., Yang, L., and Li, Q. (2014). Ulinastatin, a protease inhibitor, may inhibit allogeneic blood transfusion-associated pro-inflammatory cytokines and systemic inflammatory response syndrome and improve postoperative recovery. *Blood Transfus.* 12 Suppl 1, s109–s118. doi: 10.2450/2013.0224-12

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., and Bauer, M. (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). *Jama* 315 (8), 801–810. doi: 10.1001/jama.2016.0287

Song, J. E., Kang, W. S., Kim, D. K., Yoon, T. G., Kim, T. Y., and Bang, Y. S. (2011). The effect of ulinastatin on postoperative blood loss in patients undergoing open heart surgery with cardiopulmonary bypass. *J. Int. Med. Res.* 39 (4), 1201–1210. doi: 10.1177/147323001103900408

Stoller, J., Halpin, L., Weis, M., Aplin, B., Qu, W., and Georgescu, C. (2016). Epidemiology of severe sepsis: 2008-2012. *J. Crit. Care* 31 (1), 58–62. doi: 10.1016/j.jcrc.2015.09.034

Sun, R., Zhang, Y., Lv, Q., Liu, B., Jin, M., and Zhang, W. (2011). Toll-like receptor 3 (TLR3) induces apoptosis via death receptors and mitochondria by up-regulating the transactivating p63 isoform alpha (TAP63alpha). *J. Biol. Chem.* 286 (18), 15918–15928. doi: 10.1074/jbc.M110.178798

Sung, W. M., Sung, W. L., Yun, S. H., Dae, W. P., Ik, J. J., and Young, H. Y. (2009). The effects of urinary trypsin inhibitor on the outcomes of severe sepsis and septic shock patients. *Korean Emergency Medical J.* 20, 80–85.

Tang, J. (2013). The clinical effect analysis of treating septicopyemia patients with ulinastatin. *Guide China Med.* (15), 439–440. doi: 10.3969/j.issn.1671-8194.2013.15.333

Uchida, M., Abe, T., Ono, K., and Tamiya, N. (2018). Ulinastatin did not reduce mortality in elderly multiple organ failure patients: a retrospective observational study in a single center ICU. *Acute Med. Surg.* 5 (1), 90–97. doi: 10.1002/ams2.304

Wang, Y., Li, X., and Kexi, L. (2007). Influence of Ulinastatin on cytokines of patients with sepsis. *Modern Med. J. Of China* 9 (11), 23–25. doi: 10.3969/j.issn.1672-9463.2007.11.008

Wang, F. Y., Fang, B., Qiang, X. H., Yu, T. O., Zhong, J. R., Cao, J., et al. (2016). The efficacy and immunomodulatory effects of ulinastatin and thymosin alpha1 for sepsis: a systematic review and meta-analysis. *Biomed. Res. Int.* 2016, 9508493. doi: 10.1155/2016/9508493

Wiedow, O., and Meyer-Hoffert, U. (2005). Neutrophil serine proteases: potential key regulators of cell signalling during inflammation. *J. Intern. Med.* 257 (4), 319–328. doi: 10.1111/j.1365-2796.2005.01476.x

Wong, W. W. (1998). ICE family proteases in inflammation and apoptosis. *Agents Actions* Suppl 49, 5–13. doi: 10.1007/978-3-0348-8857-8_2

Wu, J., Zhou, L., Liu, J., Ma, G., Kou, Q., and He, Z. (2013a). The efficacy of thymosin alpha 1 for severe sepsis (ETASS): a multicenter, single-blind, randomized and controlled trial. *Crit. Care* 17 (1), R8. doi: 10.1186/cc11932

Wu, T. J., Zhang, L. N., and Kang, C. C. (2013b). The effect of ulinastatin on disbalance of inflammation and immune status in patients with severe sepsis. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue* 25 (4), 219–223. doi: 10.3760/cma.j.issn.2095-4352.2013.04.010

Wu, Y., Cui, Z. B., Kang, H. S., Zhao,Y. Y. and Liu, S. H. (2016). Effect of ulinastatin on the serum levels of tumor necrosis factor α, interleukin 10,troponin I and C-reactive protein. *J. Bengbu Med. College* 41 (12), 1635–1638. doi: 10.13898/j.cnki.issn.1000-2200.2016.12.031

Xu, Q., Yan, Q., and Chen, S. (2018). Ulinastatin is effective in reducing mortality for critically ill patients with sepsis: a causal mediation analysis. *Sci. Rep.* 8 (1), 14360. doi: 10.1038/s41598-018-32533-9

Yang, H., Mao, Y., Lu, X., Sang, X., Du, S., and Zhao, H. (2011). The effects of urinary trypsin inhibitor on liver function and inflammatory factors in patients undergoing hepatectomy: a prospective, randomized, controlled clinical study. *Am. J. Surg.* 202 (2), 151–157. doi: 10.1016/j.amjsurg.2010.07.044

Zhang, Y., Zhou, Y., Lou, J., Li, J., Bo, L., and Zhu, K. (2010). PD-L1 blockade improves survival in experimental sepsis by inhibiting lymphocyte apoptosis and reversing monocyte dysfunction. *Crit. Care* 14 (6), R220. doi: 10.1186/cc9354

Zhang, H., Tan, C., Wang, X., Kang, D., Chen, Y., and Xiong, J. (2016). Preventive effects of ulinastatin on complications related to pancreaticoduodenectomy: a Consort-prospective, randomized, double-blind, placebo-controlled trial. *Med. (Baltimore)* 95 (24), e3731. doi: 10.1097/md.0000000000003731

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CORRECTION
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Corrigendum: Improvement of Sepsis Prognosis by Ulinastatin: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

Huifang Wang[1†], Bin Liu[2†], Ying Tang[1†], Ping Chang[1], Lishuai Yao[3], Bo Huang[1], Robert F. Lodato[4] and Zhanguo Liu[1]*

[1] Department of Intensive Care Unit, Zhujiang Hospital, Southern Medical University, Guangzhou, China, [2] Emergency Department, Zhujiang Hospital, Southern Medical University, Guangzhou, China, [3] Department of Thoracic and Cardiovascular Surgical, Zhujiang Hospital, Southern Medical University, Guangzhou, China, [4] Department of Pulmonary, Critical Care, and Sleep Medicine, Medical School, University of Texas Health Science Center at Houston, Houston, TX, United States

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Min Yang,
Anhui Medical University, China

***Correspondence:**
Zhanguo Liu
zhguoliu@163.com

[†]These authors have contributed equally to this work

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Keywords: sepsis, ulinastatin, mortality, inflammatory cytokine, immune system

A Corrigendum on

Improvement of Sepsis Prognosis by Ulinastatin: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

by Wang H, Liu B, Tang Y, Chang P, Yao L, Huang B, Lodato RF and Liu Z. (2019). Front. Pharmacol.10:1370 doi: 10.3389/fphar.2019.01370

In the original article, there was a mistake in **Figure 3** as published. In **Figure 3**, we found that some data of TNF-a in **Figure 3B** was copied to **Figure 3A** by mistake. We reanalyzed the data and the new **Figure 3A** was generated. In **Figure 3C**, the left graph label should be "Control" and the right label should be "UTI." This mistake was made because the system default label was not changed when using the software. The corrected **Figure 3** appears below.

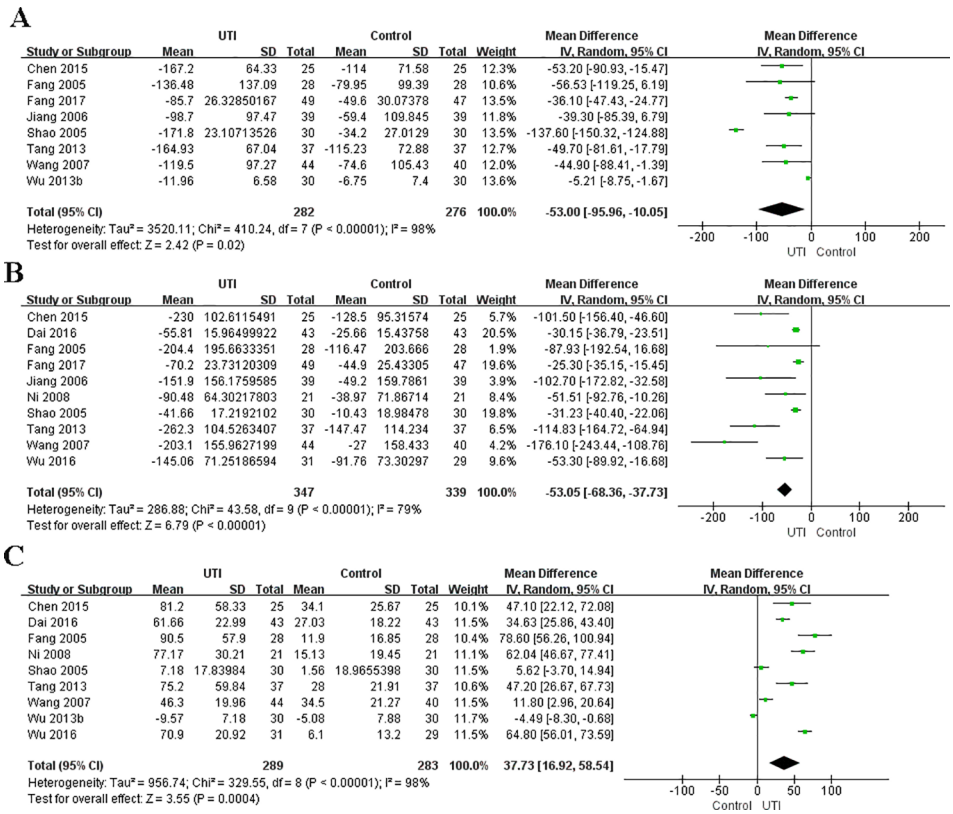

FIGURE 3 | Ulinastatin regulates the levels of pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory factor: IL-6 **(A)**, TNF-α **(B)**. Anti-inflammatory factors: IL-10 **(C)**.

In **Figure 4**, Karnad et al., 2014 was mistakenly added instead of Wu et al., 2016. We reanalyzed the data and the new **Figure 4** was generated. The corrected **Figure 4** appears below.

Additionally, Fang and Zhao, 2017; Choudhuri et al., 2015; Shao et al., 2005; Pavan Kumar et al., 2017 were not cited in the article. The citation has now been inserted in the section **Results**, subsection **Description of Eligible Studies**, paragraph 1 and should read:

"We identified 15 (Fang and Chen, 2005; Jiang et al., 2006; Wang et al., 2007; Ni et al., 2008; Sung et al., 2009; Tang, 2013; Wu et al., 2013b; Karnad et al., 2014; Chen et al., 2015; Dai and Wang, 2016; Wu et al., 2016; Fang and Zhao, 2017; Choudhuri et al., 2015; Shao et al., 2005; Pavan Kumar et al., 2017) potential studies that included a total of 1358 patients: 630 patients in the UTI group and 728 patients in the control group. Thirteen RCTs and two prospective studies were included in this meta-analysis. The specific method for identifying studies and establishing the inclusion and exclusion criteria is shown in **Figure 1**. Eleven studies were published from China, three from India, and one from Korea."

The reference for Fang et al., 2005 was incorrectly written as Karnad et al., 2014. The citation has now been inserted in the **Results** section, subsection **The APACHE Ⅱ Score**, paragraph 1. The corrected paragraph appears below:

"The APACHE II score at the time of hospital admission were not different between the UTI and control groups. We extracted the data from four studies (Ni et al., 2008; Fang et al., 2005;

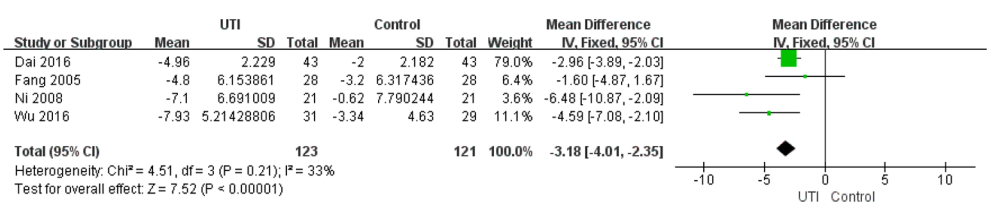

FIGURE 4 | Ulinastatin reduces the APACHE II score of sepsis patients.

Dai and Wang, 2016; Wu et al., 2016) (244 participants in two groups) to assess this change. After treatment, the APACHE II scores were significantly less in the UTI group than in the control group MD = -3.18, 95%CI [-4.01, -2.35], p < 0.00001), and heterogeneity was low (x^2 = 4.51, p = 0.21, I^2 = 33%). The results are shown in **Figure 4**".

In the **Results** section, subsection **Publication Bias and Sensitivity Analysis**, paragraph 2, the first citation of Wang et al. (2007) should be Wu et al. (2013b). The corrected paragraph appears below:

"Sensitivity analysis was conducted by the leave-one-out method and checking the consistency of the overall effect estimate. For IL-6, we found that the I^2 value decreased to 0% after excluding the studies conducted by Shao et al. (2005) and Wu et al. (2013b). For TNF-α, we found that the I^2 value decreased to 54% after excluding the studies conducted by Tang et al. (2013) and Wang et al. (2007). For IL-10, we found that the I^2 value decreased to 84% after removing the study by Shao et al. (2005), Wang et al. (2007) and Wu et al. (2013b). We believe that the high heterogeneity may arise from factors such as sample size, different measuring instruments, and design methods".

In the abstract, "(MD = -88.5, 95% CI [-123.97,-53.04], p < 0.00001)" of IL-6 were changed to "MD = -53.00, 95% CI [-95.56, -10.05], p = 0.02." "95% confidence interval (CI) [0.35-0.66]" of all-cause mortality was changed to "95% confidence interval (CI) [0.35, 0.66]". "mean difference (MD) = -2.40, 95% CI [-4.37, -0.44], p = 0.02, I^2 = 66%" of APACHE II score was changed to "mean difference (MD) = -3.18, 95% CI [-4.01, -2.35], p < 0.00001, I^2= 33%". "MD = -56.22, 95% CI [-72.11, -40.33], p < 0.00001" of TNF-α was changed to "MD = -53.05, 95%CI [-68.36, -37.73], p < 0.00001". The mistake was correlated with the mistake of **Figure 3A**; it has been changed in the corresponding place in the text. A correction has been made to the **Abstract**:

"Results: Ulinastatin significantly decreased the all-cause mortality {odds ratio (OR) = 0.48, 95% confidence interval (CI) [0.35, 0.66], p < 0.00001, I^2 = 13%}, Acute Physiology, Age, Chronic Health Evaluation II (APACHE II) score {mean difference (MD) = -3.18, 95%CI [-4.01, -2.35], p < 0.00001, I^2 = 33%, and reduced the incidence of multiple organ dysfunction syndrome (MODS) (OR = 0.3, 95% CI [0.18, 0.49], p < 0.00001, I^2 = 0%). Ulinastatin also decreased the serum levels of IL-6 (MD = -53.00, 95% CI [-95.56,-10.05], p = 0.02), TNF-α MD = -53.05, 95%CI [-68.36, -37.73], p < 0.00001, and increased the serum levels of IL-10 (MD = 37.73, 95% CI [16.92, 58.54], p = 0.0004). Ulinastatin administration did not lead to any difference in the occurrence of adverse events."

In the subsection *Outcomes and Data Extraction*, the formula "$X = |X_2 - X_1|$" was changed to "$X = X_2 - X_1$." The reason for this correction is that the observation index in this study was the effect of ulinastatin for sepsis patients. "X_2 represents the endpoint SD. R = 0.5" was changed to "S_2 represents the endpoint SD. R = 0.5." The mistakes were made due to a typographical error. A correction has been made to the **Materials and Methods** section, subsection **Outcomes and Data Extraction**, paragraph 1:

"The primary outcome was all-cause mortality. The secondary outcomes were changes in the serum levels of IL-6, IL-10, and TNF-α, the incidence rate of MODS, and changes in Acute Physiology, Age, Chronic Health Evaluation II (APACHE II) scores. We also collected the following information: study design, year of study, country, study period, the number of patients included, intervention methods, and adverse events. The main characteristics of the included studies are shown in **Table 2**. For the continuous variables, we acquired data according to the following method. For calculating the mean in this meta-analysis, we employed the formula $X = X_2 - X_1$, where X represents the mean applied in this meta-analysis, X_1 represents the baseline mean, and X_2 represents the endpoint mean. For calculating the standard deviation (SD) in this meta-analysis, we chose to employ the formula $S^2 = S_1^2 + S_2^2 - 2 \times R \times S_1 \times S_2$, where S represents the standard deviation applied in this meta-analysis, S_1 represents the baseline SD, and S_2 represents the endpoint SD. R = 0.5 in the meta-analysis, which was described in the Cochrane Handbook. All data were independently extracted by two authors (HW and LY). HW entered data into the computer and LY checked them."

In the subsection "All-Cause Mortality," p = 0.37 was changed to p = 0.32. The mistake was made due to a typographical error. A correction has been made to the **Results** section, subsection **Primary Outcomes, All-Cause Mortality**, paragraph 1:

"We extracted the data from 12 studies (Fang and Chen, 2005; Shao et al., 2005; Ni et al., 2008; Sung et al., 2009; Tang, 2013; Wu et al., 2013b; Karnad et al., 2014; Chen et al., 2015; Choudhuri et al., 2015; Wu et al., 2016; Fang and Zhao, 2017; Pavan Kumar Rao et al., 2017) and 1110 participants were classified into two groups to assess all-cause mortality. All-cause mortality was significantly lower in the UTI group than in the control group (OR = 0.48, 95% CI [0.35, 0.66], p < 0.00001), and heterogeneity was low (x^2 = 12.57, p = 0.32, I^2 = 13%). The results are shown in **Figure 2**."

In the subsection *Levels of IL-6*, the 402 participants in two groups was changed to 558 participants in two groups. The mistake was made due to a miscalculation. "MD = -88.50, 95% CI [-123.97, -53.04], p < 0.00001" was changed to "MD = -53.00, 95% CI [-95.56,-10.05], p = 0.02"; "x^2 = 249.27, p < 0.00001, I^2 = 96%" was changed to "x^2 = 410.24, p < 0.00001, I^2 = 98%." The error was made due to the mistake in **Figure 3A**. A correction has been made to the **Results** section, subsection **Levels of IL-6**, paragraph 1:

"We obtained the related data from eight studies (Fang and Chen, 2005; Shao et al., 2005; Jiang et al., 2006; Wang et al., 2007; Tang, 2013; Wu et al., 2013b; Chen et al., 2015; Fang and Zhao, 2017) (558 participants in two groups) to analyze the serum levels of IL-6. The serum level of IL-6 at the time of hospital admission was not different between the UTI and control groups. After treatment, IL-6 was significantly less in the UTI group than in the control group (MD = -53.00, 95% CI [-95.56,-10.05], p = 0.02), and a obvious heterogeneity in the results was observed (x^2 = 410.24, p < 0.00001, I^2 = 98%). The results are shown in **Figure 3A**."

In the subsection of *Levels of TNF-a*, "MD = −56.22, 95% CI [−72.11, −40.33], p < 0.00001" was changed to "MD = -53.05,

95%CI [-68.36,-37.73], p < 0.00001"; "x^2 = 47.26, p < 0.00001, I^2 = 81%" was changed to "x^2 = 43.58, p < 0.00001, I^2 = 79%." A correction has been made to the **Results** section, subsection **Levels of TNF-a**, paragraph 1:

"We collected the related data from ten studies (Fang and Chen, 2005; Shao et al., 2005; Jiang et al., 2006; Wang et al., 2007; Ni et al., 2008; Tang, 2013; Chen et al., 2015; Dai and Wang, 2016; Wu et al., 2016; Fang and Zhao, 2017) (686 participants in two groups) to analyze the serum levels of TNF-α. The level of TNF-α at the time of hospital admission was not different between the UTI and control groups. After treatment, TNF-α was significantly less in the UTI group than in the control group (MD = -53.05, 95%CI [-68.36,-37.73], p < 0.00001), and an obvious heterogeneity was observed in the results (x^2 = 43.58, p < 0.00001, I^2 = 79%). The results are shown in **Figure 3B**."

In the subsection *The Apache Ii Score*, the title should be *The Apache Ⅱ score*. Also in this section, the (298 participants in two groups) was changed to (244 participants in two groups). "MD = −2.40, 95% CI [−4.37, −0.44], p = 0.02" was changed to "MD = -3.18, 95%CI [-4.01,-2.35], p < 0.00001"; "x^2 = 8.86, p = 0.03, I^2 = 66%" was changed to "x^2 = 4.51, p = 0.21,I^2 = 33%." The mistake was made by including wrong citation. A correction has been made to the section **Results**, subsection **The Apache Ⅱ Score**, **Title**: **The Apache Ⅱ Score**, paragraph 1:

"The APACHE II scores at the time of hospital admission were not different between the UTI and control groups. We extracted the data from four studies (Ni et al., 2008; Fang et al., 2005; Dai and Wang, 2016; Wu et al., 2016) (244 participants in two groups) to assess this change. After treatment, the APACHE II scores were significantly less in the UTI group than in the control group (MD = -3.18, 95%CI [-4.01,-2.35], p < 0.00001), and heterogeneity was low (x^2 = 4.51, p = 0.21,I^2 = 33%). The results are shown in **Figure 4**."

In the section of **Publication Bias and Sensitivity Analysis**, "74%" was changed to "0%." "81%" was changed to "54%." The mistake was made by included wrong study. Also, we deleted the sensitivity for APACHE II score and added IL-10. Because we found that the heterogeneity of APACHE II was 33% and the heterogeneity of IL-10 was 98%. A correction has been made to **Results** section, subsection **Publication Bias and Sensitivity Analysis**, paragraph 2:

"Sensitivity analysis was conducted by the leave-one-out method and checking the consistency of the overall effect estimate. For IL-6, we found that the I^2 value decreased to 0% after excluding the studies conducted by Shao et al. (2005) and Wu et al. (2013b). For TNF-α, we found that the I^2 value decreased to 54% after excluding the studies conducted by Tang et al. (2013) and Wang et al. (2007). For IL-10, we found that the I^2 value decreased to 84% after removing the study by Shao et al. (2005), Wang et al. (2007) and Wu et al. (2013b). We believe that the high heterogeneity may arise from factors such as sample size, different measuring instruments, and design methods."

The authors apologize for these errors and state that they do not change the scientific conclusions of the article in any way. The original article has been updated.

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Six-Year Immunologic Recovery and Virological Suppression of HIV Patients on LPV/r-Based Second-Line Antiretroviral Treatment: A Multi-Center Real-World Cohort Study in China

Xiaojie Huang[1†], Liumei Xu[2†], Lijun Sun[1], Guiju Gao[3], Weiping Cai[4], Yanfen Liu[5], Haibo Ding[6], Hongxia Wei[7], Ping Ma[8], Min Wang[9], Shuiqing Liu[10], Yaokai Chen[11], Xiaohong Chen[12], Qingxia Zhao[13], Jianhua Yu[14], Yuxia Song[15], Hui Chen[16], Hao Wu[1], Shanfang Qin[17] and Linghua Li[4]**

[1] Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, China, [2] Department of Clinical AIDS Research, the Third People's Hospital of Shenzhen, Shenzhen, China, [3] Clinical and Research Center for Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [4] InInfectious Diseases Center, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China, [5] Center for Infectious Diseases, the Fourth People's Hospital of Nanning, Nanning, China, [6] NHC Key Laboratory of AIDS Immunology (China Medical University), Department of Laboratory Medicine, The First Affiliated Hospital of China Medical University, Shenyang, China, [7] Department of Infectious Disease, The Second Hospital of Nanjing, Affiliated Nanjing Hospital of Nanjing University of Chinese Medicine, Nanjing, China, [8] Department of Infectious Disease, the Affiliated Second Peoples' Hospital of the Nankai University, Tianjin, China, [9] Institute of HIV/ AIDS, The First Hospital of Changsha, Changsha, China, [10] Department of Infectious Diseases, Guiyang Public Health Clinical Center, Guiyang, China, [11] Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing, China, [12] Department of Infectious Diseases, the Fourth Affiliated Hospital of Harbin Medical University, Harbin, China, [13] Department of Infectious Diseases, Henan Infectious Disease Hospital, Zhengzhou, China, [14] Department of Infectious Diseases, XIXI Hospital of Hangzhou, Hangzhou, China, [15] Department of Infectious Diseases, Xinjiang Uygur Autonomous Region Sixth People's Hospital, Xinjiang, China, [16] School of Biomedical Engineering, Capital Medical University, Beijing, China, [17] Department of Infectious Diseases, Longtan Hospital of Guangxi Zhuang Autonomous Region, Liuzhou, China*

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Edited by:
Yonggang Zhang, Sichuan University, China

Reviewed by:
Tauqeer Hussain Mallhi, University of Science Malaysia, Malaysia
Paul Palumbo, Dartmouth–Hitchcock Medical Center, United States

***Correspondence:**
Shanfang Qin
qsf2271976481@163.com
Linghua Li
llheliza@126.com

†These authors have contributed equally to this work

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The World Health Organization guidelines recommend lopinavir/ritonavir (LPV/r) as a second-line antiretroviral therapy (ART) for HIV-infected adults in middle-income and low-income countries as a protease inhibitor boost based on clinical trials; however, the real-world safety and efficacy remain unknown. Therefore, we conducted a large-scale, multicenter retrospective cohort study to evaluate the efficacy and safety of LPV/r-based ART among HIV-infected adults in China in whom first-line therapy failed. The data were obtained from a national database covering 17 clinics in China for six years of follow-up from 2009 to 2016. Failure of first-line treatment was determined according to a viral load at least 400 copies/ml at week 48, non-completers at week 48 for any reason, and those who switched ART before week 48 for any reason such as side effects. Treatment effectiveness was assessed by the rate of CD4$^+$T cell recovery, defined as >500 cells/mm^3, and the proportion of patients achieving viral suppression, defined as <400 or <50 copies/ml according to the methods used during treatment. Safety was assessed by rates of LPV/r-related adverse events (AEs), including lipid disorder, severe abnormal liver function, myelosuppression, and renal function. Between 2009 and 2016, 1196 participants (median, 36 years old; IQR, 30–43 years) were ultimately enrolled. All patients

had been on LPV/r-based second-line ART treatment for more than one year after failure of any first-line ART regimen. Overall CD4+T cell counts increased from 138 cells/mm^3 to 475 cells/mm^3 and 37.2% of all participants reached CD4 recovery. Viral suppression rates dramatically increased at the end of the first year (<400 copies/ml, 88.8%; <50 copies/ml, 76.7%) and gradually increased during follow-up (<400 copies/ml, 95.8%; <50 copies/ml, 94.4%). The most frequently reported AEs were LPV/r-induced lipid disorders with no obvious increase on LDL-C at follow-up visits. This is the first real-world LPV/r-based second-line treatment study to cover such a large population in China. These results provide strong clinical evidence that LPV/r-based second-line ART is effective in increasing CD4+T cell counts and viral suppression rates with tolerable side effects in HIV-infected adults in China in whom first-line treatment had failed.

Keywords: efficacy and safety, ART-experienced, second-line antiretroviral therapy, human immunodeficiency virus, lopinavir/ritonavir

INTRODUCTION

Over two thirds of new cases of HIV diagnosed globally in 2017 were estimated to have occurred in resource-limited areas, including eastern and southern Africa, western and central Africa, and Latin America (UNAIDS, 2018). Thousands of HIV-infected adults who live in these resource-limited countries nevertheless have access to antiretroviral therapy (ART) according to World Health Organization (WHO) guidelines (Gilks et al., 2006), which has largely contributed to the reduction in mortality and morbidity associated with HIV infection and has remarkably improved quality of life of people living with HIV/AIDS (PLWHA) (Mills et al., 2011; Johnson et al., 2012; Teeraananchai et al., 2017).

However, an increasing number of HIV-infected adults have shown first-line regimen failure, requiring a switch to second-line therapy (Fox et al., 2012; Liégeois et al., 2012). Moreover, a recent study presented at the 25th Conference on Retroviruses and Opportunistic Infections (CROI 2018) demonstrated that more than half of all HIV-infected adults in low- and middle-income countries may not achieve and maintain continuous viral suppression under second-line ART. Thus, it is essential for clinicians to assess the optimum second-line ART regimen in PLWHA in resource-constrained areas in whom first-line therapy has failed.

The WHO guidelines recommend second-line combination ART with a ritonavir-boosted protease inhibitor (PI; either lopinavir or atazanavir) combined with at least two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). Lopinavir/ritonavir (LPV/r) is currently widely used in middle-income and low-income countries, such as China and South Africa, based on demonstrated effectiveness and safety with respect to immunological restoration and tolerable side-effects in ART-naïve and experienced patients in combination with other ART drugs in clinical trials (Cohen et al., 2005; Paton et al., 2014; Ciaffi et al., 2015; La Rosa et al., 2016). Currently, boosted PI options are recommended as part of second-line regimens because of their safety and efficacy as indicated by systematic reviews and meta-analyses (Hermes et al., 2012; Huang et al., 2018). However, there is still no solid real-world evidence for the long-term safety and efficacy of LPV/r as second-line therapy in resource-limited settings.

Many factors can potentially influence real-world efficacy (Sherman et al., 2016), including adherence, the first-line ART regimen, baseline CD4 counts, viral load, and age, before switching to second-line treatment, which could limit the external validity of traditional randomized clinical trials (RCTs). Furthermore, the present WHO guideline was based on the results of RCTs in limited countries; thus, more evidence on efficacy is required to support decisions on treatment (Wang et al., 2009; Sherman et al., 2016). As a result, there is an urgent need for more data on the long-term real-world efficacy of this widely used second-line regimen to enable clinicians to make informed judgements for patient selection in resource-constrained areas for whom first-line therapy has failed.

Therefore, we conducted the present Chinese multi-center real-world cohort study to provide suitable data on the efficacy and safety of second-line ART with LPV/r for all patients in whom first-line ART had failed, which can help develop standard guidelines for treatment.

METHODS

Study Design and Participants

This large-scale multi-center retrospective study was conducted using data collected from a national database from 2009 to 2016 across 17 clinics in China (Jing et al., 2017). This study was reviewed and approved by the Beijing Youan Hospital institutional board, which was the leading research institute for this study. Eligibility criteria for included participants were as follows: 1) adults 18 years or older, and 2) failure of any first-line ART regimen (viral load of least 400 copies/ml at week 48), followed by LPV/r-based second-line ART. In brief, each center collected data from existing national databases on demographics (age, gender), baseline

information (CD4+T cell counts, viral load, and WHO stage), ART treatment history, and HIV-related diseases. There was no adverse events (AEs) information in the national databases; therefore, details on AEs were collected separately according to participants' medical records.

Procedures

The efficacy of the second-line LPV/r regimen was evaluated according to the immunological and virological responses at baseline and at 6, 12, and 72 months on ART.

Good immune recovery was defined as a CD4+T cell count more than 500 cells/mm^3, and the percentage of viral suppression was defined as a viral load below 50 or 400 copies/ml. WHO-defined stage IV disease was determined according to the WHO clinical staging of HIV disease in adults. Factors related to recovery of CD4+T cell counts and WHO-defined stage IV disease were also analyzed. The safety of second-line LPV/r regimens was evaluated according to the rates of drug-related AEs (see **Table S1** for definitions of each AE). An AE was considered if any result in the follow-up visit was abnormal.

Statistical Analysis

Baseline demographic and clinical data were stratified according to the baseline age of participants (<50 years or ≥50 years). Descriptive statistics are presented as medians with interquartile ranges (IQRs) or counts with proportions as appropriate. Two-sample *t*-tests were used to compare means, and χ^2 tests were used to compare proportions. Fisher's exact test was used when there were fewer than 40 participants or when expected values were lower than 1 in 20% of the cells for R × C tables. Correction for continuity was performed when there were more than 40 participants and expected values were between 1 and 5 in 20% of the cells for R × C tables. The Cox proportional-hazards model was used to investigate associations between baseline levels of CD4+ T cells, viral load, or age of participants and good immune reconstitution. Factors associated with baseline WHO-defined Stage IV disease were identified by logistic regression model. Risk ratios (RRs) for good immune recovery were estimated with 95% confidence intervals (CIs). All *P* values were two-sided, and $P < 0.05$ was considered statistically significant. The data were analyzed using SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Participant Selection and Baseline Characteristics

Between 2009 and 2016, 4006 patients used LPV/r as a second-line drug. Among these, 2078 patients with a viral load below 400 copies/ml at baseline were excluded from the study, leaving a total of 1928 patients in the first-line treatment failure group. A further 53 patients below 18 years of age, 556 patients who were undergoing the second-line treatment for less than 1 year, and 23 duplicate records were excluded, resulting in a total 1196 participants who failed any first-line ART regimen and switched to LPV/r-based second-line ART enrolled in the study.

The median age of the eligible participants was 36 years (IQR, 30–43 years). There were fewer co-infections with hepatitis C virus among those older than 50 years than among the younger patients. Distributions of routes of transmission also significantly differed among the two age groups ($P < 0.001$). Detailed information of the other differences in baseline characteristics according to age group is shown in **Table 1**.

TABLE 1 | Baseline characteristics of included participants

	Age ≤ 50 years (n = 1034)	Age > 50 years (n = 121)	Overall[#] (n = 1155)	*P* value
Male gender, n (%)	862 (83.4%)	100 (82.6%)	962 (83.3%)	0.841
Time since HIV diagnosis (months) median (IQR)	24 (15,39)	21 (12,39)	24 (15,39)	0.434
Route of infection				<0.001
Blood transfusion	19 (1.8%)	1 (0.8%)	20 (1.7%)	
Plasma	5 (0.5%)	1 (0.8%)	6 (0.5%)	
Drug injection	171(16.5%)	4 (3.3%)	175 (15.2%)	
Homosexual sexual transmission	340 (32.9%)	23 (19.0%)	363 (31.4%)	
Heterosexual sexual transmission	430 (41.6%)	80 (66.1%)	510 (44.2%)	
Other	69 (6.7%)	12 (9.9)	81 (7.0%)	
Co-infection with HCV, n (%)	66 (6.4%)	2 (1.7%)	68 (5.9%)	0.036
Co-infection with HBV, n (%)	66 (6.4%)	5(4.1%)	71 (6.1%)	0.329
HIV-1 RNA (log copies/ml), median (IQR)	4.4 (3.7–5.0)	4.4 (3.8–5.1)	4.4 (3.7–5.0)	0.500
HIV-1 RNA < 3	43 (4.2%)	3 (2.5%)	46 (4.0%)	0.517
HIV-1 RNA ≥ 3	991 (95.8%)	118 (97.5%)	1109 (96.0%)	
Baseline CD4+T-cell count (cells/mm^3), median (IQR)	141 (57–265)	119 (48–225)	138 (54–262)	0.095
Baseline CD4+T-cell count < 350	891 (86.2%)	108 (89.3%)	999 (86.5%)	0.385
Baseline CD4+T-cell count ≥ 350	140 (13.5%)	13 (10.7%)	153 (13.2%)	
Missing	3 (0.3%)	0	3 (0.3%)	

[#]*41 participants were missing baseline data on age.*

CD4+T Cell Counts Recovery

Figure 1A shows the dramatically increasing trend of median CD4+T cell counts over the six years of LPV/r treatment. Patients with baseline CD4+T cell counts > 350 cells/mm^3 showed significantly higher immunological recovery and a lower WHO-defined stage IV HIV-related disease rate than those with counts below 350 cells/mm^3 (**Figure 2**). In univariate analyses, factors significantly associated with CD4 recovery included baseline CD4+T cell count, baseline viral load, and age. There was no significant association with tenofovir disoproxil fumarate (TDF)-containing treatment. In the final multivariate model, factors associated with CD4 recovery included baseline CD4+T cell count and age (**Table 2**). The age and baseline CD4+T cell count-stratified results on change trends of CD4+T cell counts are also shown in **Figure 3**.

Viral Suppression

The viral suppression rate was much higher at the end of the first year (<400 copies/ml, 88.8%, <50 copies/ml, 76.7%) and showed a slow increase during following-up (<400 copies/ml, 95.8%, <50 copies/ml, 94.4%, **Figure 1B**).

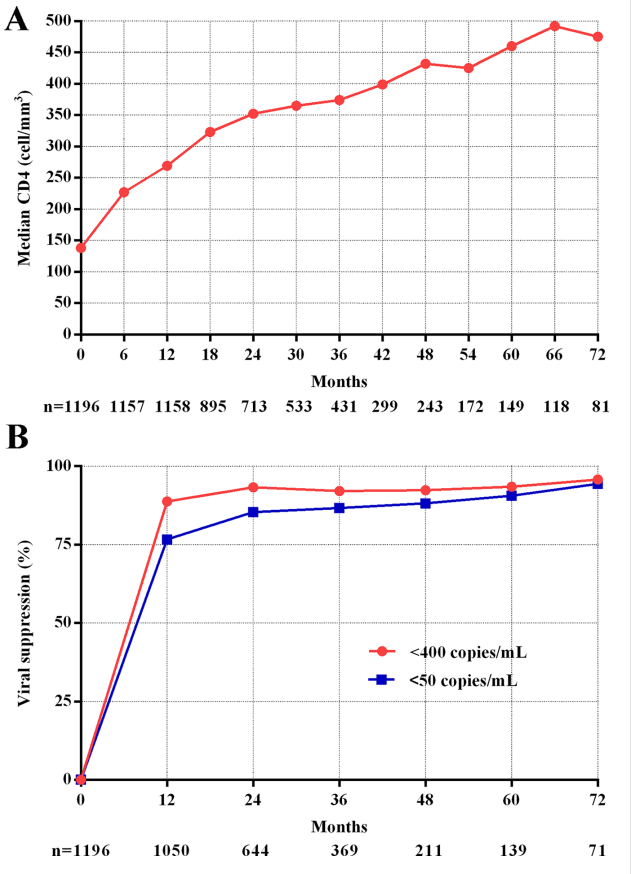

FIGURE 1 | CD4 recovery and viral suppression during follow-up. **(A)** Trend of median CD4+T cell counts. **(B)** Trend of viral suppression.

Who-Defined Stage IV HIV-Related Disease

In both univariate and multivariate analyses, baseline CD4+T cell count and baseline viral load were the only significant factors associated with baseline WHO-defined stage IV disease (**Table 3**). There was no significant association with age and TDF-containing treatment.

Adverse Events

Laboratory data of 327, 83, 364, and 306 participants related to AEs of myelosuppression, renal function, liver function, and lipid disorder were available, respectively. Over 90% of the participants had normal myelosuppression, liver function, and renal function at baseline and during follow-up. When stratified according to the baseline CD4 count, the rates of grade 3 to 4 lipid disorder and abnormal renal function for those with ≥200 cells/mm^3 were slightly higher than those in the patients with a baseline CD4 count <200 cell/mm^3. Detailed information for all AEs is shown in **Table 4** and **Figure 4**.

DISCUSSION

This study provides the first multicenter real-world evidence on the efficacy and safety of LPV/r-based second-line treatment in HIV patients across China, demonstrating that LPV/r-based ART is effective in increasing CD4+T cell counts and suppressing the viral load for patients in whom first-line treatment failed. Moreover, the patients using this second-line treatment experienced minimal drug-related AEs in this real-world setting. Age and baseline CD4+T cell counts were associated with CD4 recovery, and both the baseline CD4+T cell count and viral load were associated with viral suppression. These findings indicate that patients should be switched to second-line treatment immediately after confirmation of treatment failure.

Indeed, immunological function dramatically increased (with respect to CD4+T cell counts) in the first year after switching due to failure of first-line treatment, and remained stable during follow-up visits in this real-world setting, which is consistent with the results of previous observational studies and RCTs (Pujades-Rodríguez et al., 2008; Ferradini et al., 2011; Patel et al., 2013). Although not universally accepted, an increase of 100 cells/ml over the first year on therapy can be considered as an indicator of the success of first-line therapy given a baseline CD4+T cell count below 200 cells/mm^3 (Fox et al., 2010). Thus, the average gain of 131 cells in 1 year on second-line therapy observed in the present study represents substantial immune recovery. In addition, we found that younger age and higher baseline CD4+T cell counts favor immune recovery owing to the preservation of thymic function (Douek et al., 1998; Viard et al., 2001). With respect to the viral load, around 95% of patients under the second-line treatment regimen achieved successful viral load suppression during the six years of follow-up, which is not inferior to the efficacy of other PIs, such as DRV/r and ATV/r (Bánhegyi et al., 2012; Akanmu et al., 2015). A higher baseline CD4+T cell count and

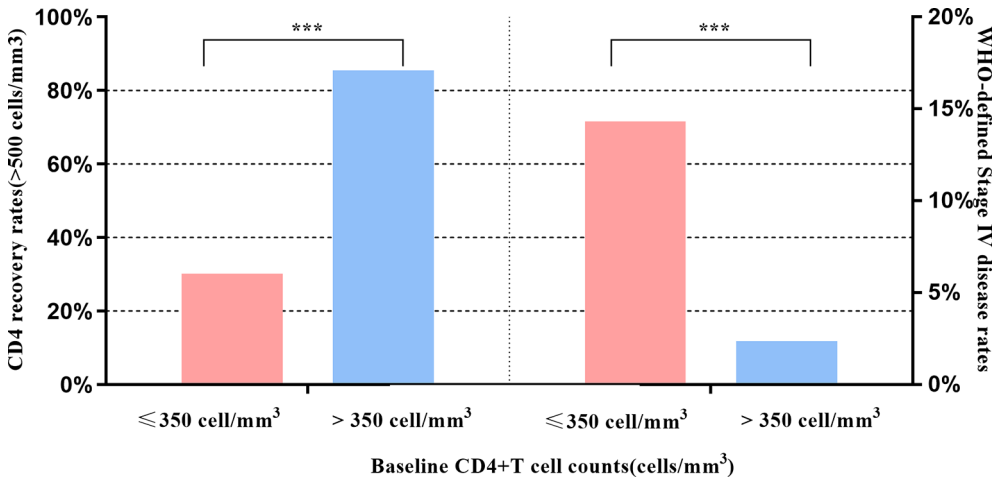

FIGURE 2 | Effects of baseline CD4 on CD4 recovery and WHO-defined stage four disease. *** indicates P < 0.001.

TABLE 2 | Factors associated with CD4 recovery.

	CD4 recovery rate n (%)	Unadjusted RR (CI)	*P* value	Adjusted RR (CI)	*P* value
Baseline CD4 > 350 cells/mm^3	136 (85.5%)	8.2 (6.62–10.17)	<0.001	8.09 (6.51–10.06)	<0.001
Baseline VL ≤ 10^5 copies/ml	370 (41.0%)	1.57 (1.23–2.02)	<0.001		0.216
Baseline age ≤ 50 years	402 (38.9%)	1.63 (1.13–2.35)	0.009	1.47 (1.02–2.12)	0.039
TDF-containing regimen	353 (41.6%)	1.22 (0.97–1.54)	0.088		0.105

RR, relative risk; CI, confidence interval; VL, viral load; TDF, tenofovir disoproxil fumarate.

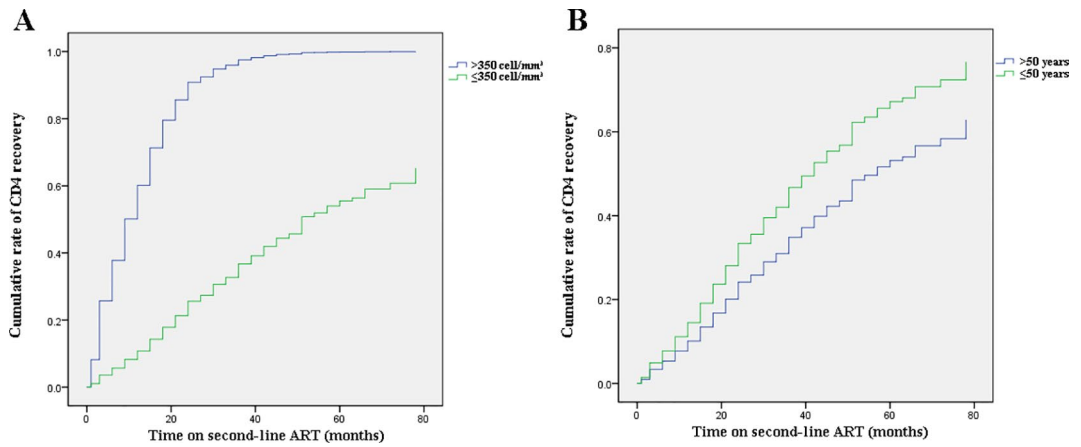

FIGURE 3 | Effects of baseline CD4+T cell counts and age on change trends of CD4+T cell counts. **(A)** Baseline CD4+T cell count-stratified results on trends of CD4+T cell counts. **(B)** Age-stratified results on trends of CD4+T cell counts.

a lower baseline viral load are protective factors for baseline HIV-related diseases. However, in resource-limited settings where ART failure is determined predominantly by clinical failure and immunological failure because viral load tests are not widely available and NRTIs resistance is very common (Hosseinipour et al., 2009). In the present cohort, HIV-1 RNA levels were routinely monitored (once per year) according to Chinese free ART guidelines, and thus the switch to the second-line regimen was likely made earlier than it would have been based only on clinical assessment.

Regarding the safety and tolerability of LPV/r-containing second-line regimens, we detected minimal LPV/r-related AEs in this real-world setting during the six-year follow-up period, which was consistent with previous cohort studies (Dlamini et al., 2011;

TABLE 3 | Factors associated with baseline WHO-defined stage IV disease.

	WHO-defined stage IV disease, n (%)	Unadjusted OR (CI)	*P* value	Adjusted OR (CI)	*P* value
Baseline CD4 > 350 cells/mm^3	3 (2.4%)	7.05 (2.20–22.57)	0.001	5.25 (1.62–16.94)	0.006
Baseline VL ≤ 10^5 copies/ml	68 (9.8%)	0.37 (0.24–0.56)	<0.001	0.42 (0.27–0.65)	<0.001
Baseline age ≤ 50 years	96 (12.2%)	0.81 (0.43–1.56)	0.536		0.623
TDF-containing regimen	79 (12.2%)	0.84 (0.55–1.29)	0.430		0.883

OR, odds ratio; CI, confidence interval; VL, viral load; TDF, tenofovir disoproxil fumarate.

TABLE 4 | Change in severity of adverse events at baseline and follow-up.

Baseline	Follow-up[#]		*P* value
	Normal	Grade 3–4	
Myelosuppression			0.012
Normal	343 (96.6%)	1 (0.3%)	
Grade 3–4	10 (2.8%)	1 (0.3%)	
Renal function			1.000
Normal	70 (100.0%)	0	
Grade 3–4	0	0	
Liver function			1.000
Normal	133 (99.3%)	1 (0.7%)	
Grade 3–4	0	0	
Blood lipid			<0.001
Normal	24 (42.9%)	16 (28.6%)	
Grade 3–4	0	16 (28.6%)	

[#]*An adverse effect was considered if any one of the follow-up visit tests was abnormal.*

Han et al., 2015). Some observational studies showed that LPV/r might be associated with the development of renal impairment, although the incidence ratios were relatively low (1.08–1.22 per year) (Mocroft et al., 2010; Ryom et al., 2013); however, we found no obvious difference in renal function compared to baseline levels. Our results highlighted that the potential for lipid disorder during LPV/r-based treatment should be carefully monitored and evaluated. Compared to LPV/r, other PIs such as DRV/r may result in a more favorable gastrointestinal and lipid profile at week 96 in spite of the non-significant discontinuation due to AEs (Mills et al., 2009). However, it is noteworthy that no obvious increase on LDL-C was found in our study, which is more relevant to the development of the atherosclerotic cardiovascular disease. In the present study, the majority of participants who had lipid disorders at baseline still had them during follow-up, which indicates that lipid profile monitoring should be integrated into standard care for patients under LPV-containing regimens.

A strength of our study is that it was performed across diverse sites in low- to middle-income areas in China with little access to

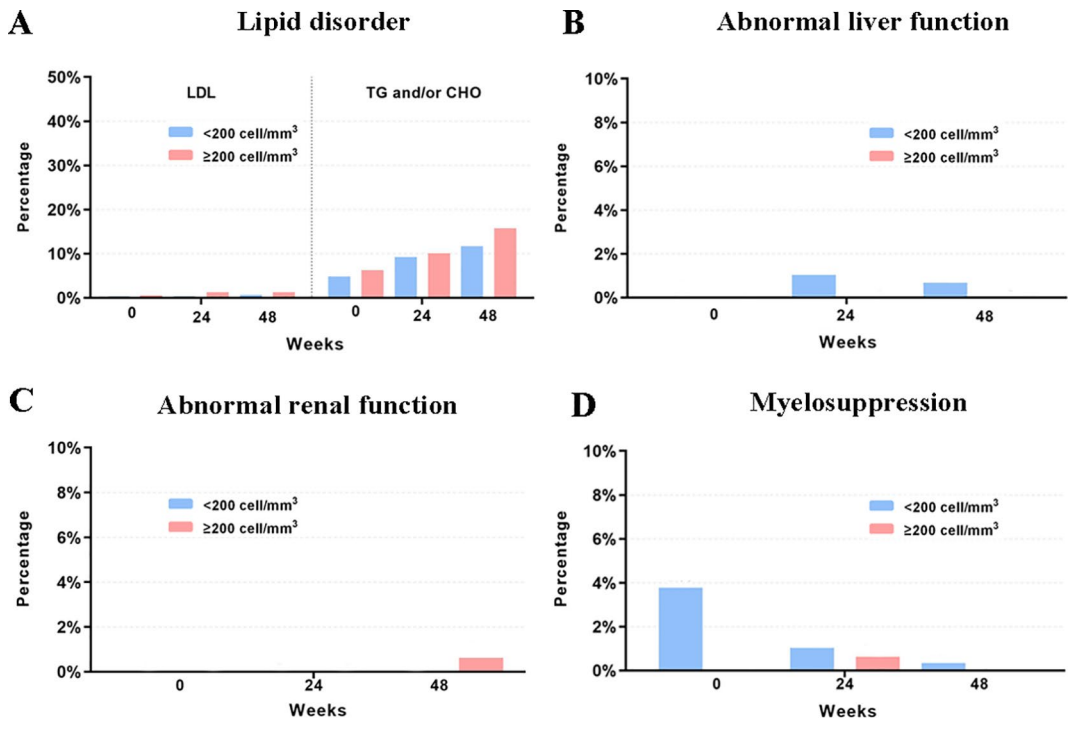

FIGURE 4 | Rates of LPVr related AEs across baseline, week 24 and week 28. **(A)** The rates of grade 3 to 4 lipid disorder. **(B)** The rates of grade 3 to 4 abnormal liver function. **(C)** The rates of grade 3 to 4 abnormal renal function. **(D)** The rates of grade 3 to 4 myelosuppression. LDL, low-density lipoprotein. TG, triglycerides. CHO, cholesterol.

other PIs, and using a real-world study design. Thus, these results can be generalizable to settings where the majority of people with HIV reside. One weakness of this study is that the retrospective design and missing data for some baseline characteristics and outcomes could have contributed to bias, which calls for caution when interpreting causal relationships for some analyses. In addition, the definition of AE as any abnormal finding during follow-up visit tests might have resulted in an overestimation of the AE prevalence. However, the use of large real-world samples from different provinces across China could improve the representativeness including participants with different demographic backgrounds, along with the higher statistical power to detect potentially significant effects.

CONCLUSION

In summary, this national multicenter study contributes clear and generalizable findings to real-world settings and provides solid evidence of the suitability of LPV/r as second-line ART in resource-limited countries. Our data support the current WHO recommendation for a boosted PI plus NRTIs as second-line HIV therapy after failure of non-NRTI-based regimens in resource-limited settings.

DATA AVAILABILITY STATEMENT

Raw data is available upon request to the first author. Requests to access the datasets should be directed to Xiaojie Huang, huangxiaojie78@126.com.

ETHICS STATEMENT

This study was reviewed and approved by the Beijing Youan Hospital institutional board, which is the leading research institute for this study.

AUTHOR CONTRIBUTIONS

XH, LX, and LL led the analysis and writing of this manuscript. XH, LX, HWe, HC, HWu, SQ, and LL contributed to the final version. XH and LL designed the study. LS, GG, WC, YL, HD, PM, MW, SL, YC, XC, QZ, JY, and YS were involved in managing the data collection. All authors reviewed and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.01455/full#supplementary-material

REFERENCES

Akanmu, A. S., Adeyemo, T., Lesi, F., Bello, F. O., Okwuegbuna, K., Oloko, K., et al. (2015). Immunological and virological outcomes of patients switched from LPV/r to ATV/r-containing second-line regimens. *Curr. HIV Res.* 13, 176–183. doi: 10.2174/1570162X1303150506181434

Bánhegyi, D., Katlama, C., Arns da Cunha, C., Schneider, S., Rachlis, A., Workman, C., et al. (2012). Week 96 efficacy, virology and safety of Darunavir/r versus lopinavir/r in treatment-experienced patients in Titan. *Curr. HIV Res.* 10, 171–181. doi: 10.2174/157016212799937218

Ciaffi, L., Koulla-Shiro, S., Sawadogo, A., le Moing, V., Eymard-Duvernay, S., Izard, S., et al. (2015). Efficacy and safety of three second-line antiretroviral regimens in HIV-infected patients in Africa. *AIDS* 29, 1473–1481. doi: 10.1097/QAD.0000000000000709

Cohen, C., Nieto-Cisneros, L., Zala, C., Fessel, W. J., Gonzalez-Garcia, J., Gladysz, A., et al. (2005). Comparison of atazanavir with lopinavir/ritonavir in patients with prior protease inhibitor failure: a randomized multinational trial. *Curr. Med. Res. Opin.* 21, 1683–1692. doi: 10.1185/030079905X65439

Dlamini, J. N., Hu, Z., Ledwaba, J., Morris, L., Maldarelli, F. M., Dewar, R. L., et al. (2011). Genotypic resistance at viral rebound among patients who received lopinavir/ritonavir-based or efavirenz-based first antiretroviral therapy in South Africa. *J. Acquir. Immune Defic. Syndr.* 58, 304–308. doi: 10.1097/QAI.0b013e3182278c29

Douek, D. C., Mcfarland, R. D., Keiser, P. H., Gage, E. A., Massey, J. M., Haynes, B. F., et al. (1998). Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396, 690–695. doi: 10.1038/25374

Ferradini, L., Ouk, V., Segeral, O., Nouhin, J., Dulioust, A., Hak, C., et al. (2011). High efficacy of lopinavir/r-based second-line antiretroviral treatment after 24 months of follow up at E S T H E R/Calmette Hospital in Phnom Penh, Cambodia. *J. Int. AIDS Soc* 14, 14. doi: 10.1186/1758-2652-14-14

Fox, M. P., Ive, P., Long, L., Maskew, M., and Sanne, I. (2010). High rates of survival, immune reconstitution, and virologic suppression on second-line antiretroviral therapy in South Africa. *J. Acquir. Immune Defic. Syndr.* 53, 500–506. doi: 10.1097/QAI.0b013e3181bcdac1

Fox, M. P., Cutsem, G. V., Giddy, J., Maskew, M., Keiser, O., Prozesky, H., et al. (2012). Rates and Predictors of Failure of first-line antiretroviral therapy and Switch to second-line ART in South Africa. *J. Acquir. Immune Defic. Syndr.* 60, 428–437. doi: 10.1097/QAI.0b013e3182557785

Gilks, C. F., Crowley, S., Ekpini, R., Gove, S., Perriens, J., Souteyrand, Y., et al. (2006). The WHO public-health approach to antiretroviral treatment against HIV in resource-limited settings. *Lancet* 368, 505–510. doi: 10.1016/S0140-6736(06)69158-7

Han, Y., Li, Y., Xie, J., Qiu, Z., Li, Y., Song, X., et al. (2015). Week 120 efficacy of tenofovir, lamivudine and lopinavir/r-based second-line antiretroviral therapy in treatment-experienced HIV patients. *PloS One* 10, e0120705. doi: 10.1371/journal.pone.0120705

Hermes, A., Squires, K., Fredrick, L., Martinez, M., Pasley, M., Trinh, R., et al. (2012). Meta-analysis of the safety, tolerability, and efficacy of lopinavir/ritonavir-containing antiretroviral therapy in HIV-1-infected women. *HIV Clin. Trials* 13, 308–323. doi: 10.1310/hct1306-308

Hosseinipour, M. C., van Oosterhout, J. J. G. V., Weigel, R., Phiri, S., Kamwendo, D., Parkin, N., et al. (2009). The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. *AIDS* 23, 1127–1134. doi: 10.1097/QAD.0b013e32832ac34e

Huang, Y., Huang, X., Luo, Y., Zhou, Y., Tao, X., Chen, H., et al. (2018). Assessing the efficacy of lopinavir/ritonavir-based preferred and alternative second-line regimens in HIV-infected patients: A meta-analysis of key evidence to support WHO recommendations. *Front. Pharmacol.* 9, 890. doi: 10.3389/fphar.2018.00890

Jing, T., Lang, C., Wen, W., Yan, L., Gui, Z., and S., X. (2017). Real world study using observational designs: plan for potient registry study and development of registry database. *Chin. J. Evidenced-based Med.* 17 (12), 8. doi: 10.7507/1672-2531.201711112

Johnson, L., Mossong, J., Dorrington, R., Shomaker, M., Hoffman, C. J., Keiser, O., et al. (2012). Life expectancies of HIV-positive adults receiving antiretroviral treatment in South Africa. Actuarial Society of South Africa's 2012 Convention 16–17 October 2012, Cape Town International Conventional Centre.

La Rosa, A. M., Harrison, L. J., Taiwo, B., Wallis, C. L., Zheng, L., Kim, P., et al. (2016). Raltegravir in second-line antiretroviral therapy in resource-limited settings (SELECT): a randomised, phase 3, non-inferiority study. *Lancet HIV* 3, e247–e258. doi: 10.1016/S2352-3018(16)30011-X

Liégeois, F., Vella, C., Eymard-Duvernay, S., Sica, J., Makosso, L., Mouinga-Ondémé, A., et al. (2012). Virological failure rates and HIV-1 drug resistance patterns in patients on first-line antiretroviral treatment in semirural and rural Gabon. *J. Int. AIDS Soc* 15, 17985. doi: 10.7448/IAS.15.2.17985

Mills, A. M., Nelson, M., Jayaweera, D., Ruxrungtham, K., Cassetti, I., Girard, P. M., et al. (2009). Once-daily Darunavir/ritonavir vs. lopinavir/ritonavir in treatment-naive, HIV-1-infected patients: 96-week analysis. *AIDS* 23, 1679–1688. doi: 10.1097/QAD.0b013e32832d7350

Mills, E. J., Bakanda, C., Birungi, J., Chan, K., Ford, N., Cooper, C. L., et al. (2011). Life expectancy of persons receiving combination antiretroviral therapy in low-income countries: a cohort analysis from Uganda. *Ann. Intern. Med.* 155, 209–216. doi: 10.7326/0003-4819-155-4-201108160-00358

Mocroft, A., Kirk, O., Reiss, P., De Wit, S., Sedlacek, D., Beniowski, M., et al. (2010). Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. *AIDS* 24, 1667–1678. doi: 10.1097/QAD.0b013e328339fe53

Patel, D., Desai, M., Shah, A. N., and Dikshit, R. K. (2013). Early outcome of second line antiretroviral therapy in treatment-experienced human immunodeficiency virus positive patients. *Perspect. Clin. Res.* 4, 215–220. doi: 10.4103/2229-3485.120170

Paton, N. I., Kityo, C., Hoppe, A., Reid, A., Kambugu, A., Lugemwa, A., et al. (2014). Assessment of second-line antiretroviral regimens for HIV therapy in Africa. *N. Engl. J. Med.* 371, 234–247. doi: 10.1056/NEJMoa1311274

Pujades-Rodríguez, M., O'Brien, D., Humblet, P., and Calmy, A. (2008). Second-line antiretroviral therapy in resource-limited settings: the experience of Médecins Sans Frontières. *AIDS* 22, 1305–1312. doi: 10.1097/QAD.0b013e3282fa75b9

Ryom, L., Mocroft, A., Kirk, O., Worm, S. W., Kamara, D. A., Reiss, P., et al. (2013). Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. *J. Infect. Dis.* 207, 1359–1369. doi: 10.1093/infdis/jit043

Sherman, R. E., Anderson, S. A., Dal Pan, G. J., Gray, G. W., Gross, T., Hunter, N. L., et al. (2016). Real-world evidence - what is it and what can it tell us? *N. Engl. J. Med.* 375, 2293–2297. doi: 10.1056/NEJMsb1609216

Sherman, R. E., Anderson, S. A., Dal Pan, G. J., Gray, G. W., Gross, T., Hunter, N. L., et al. (2016). Real-World Evidence - What Is It and What Can It Tell Us? *New Engl. J. Med.* 375 (23), 2293–2297. doi: 10.1056/NEJMsb1609216

Teeraananchai, S., Chaivooth, S., Kerr, S. J., Bhakeecheep, S., Avihingsanon, A., Teeraratkul, A., et al. (2017). Life expectancy after initiation of combination antiretroviral therapy in Thailand. *Antivir. Ther. (Lond.)* 22, 393–402. doi: 10.3851/IMP3121

UNAIDS. (2018). *Global report on the global AIDS epidemic*. World Health Organization.

Viard, J. P., Mocroft, A., Chiesi, A., Kirk, O., Røge, B., Panos, G., et al. (2001). Influence of age on CD4 cell recovery in human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy: evidence from the EuroSIDA study. *J. Infect. Dis.* 183, 1290–1294. doi: 10.1086/319678

Wang, H. H., Zhou, J., He, G. P., Luo, Y., Li, X., Yang, A., et al. (2009). Consistent ART adherence is associated with improved quality of life, CD4 counts, and reduced hospital costs in Central China. *AIDS Res. Hum. Retroviruses* 25, 757–763. doi: 10.1089/aid.2008.0173

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Efficacy and Tolerability of Lopinavir/Ritonavir- and Efavirenz-Based Initial Antiretroviral Therapy in HIV-1-Infected Patients in a Tertiary Care Hospital in Beijing, China

Bin Su[1,2†], Yin Wang[3†], Ruifeng Zhou[3], Taiyi Jiang[1,2], Hongwei Zhang[1], Zaicun Li[1], An Liu[1], Ying Shao[1], Wei Hua[1], Tong Zhang[1,2], Hao Wu[1,2], Shenghua He[3], Lili Dai[1] and Lijun Sun[1]**

[1] Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, China, [2] Beijing Key Laboratory for HIV/AIDS Research, Beijing, China, [3] Center for Infectious Diseases, Public Health Clinic Center of Chengdu, Chengdu, China

Background: Lopinavir/ritonavir (LPV/r) is a major antiretroviral treatment in China, but little is known about the performance of first-line LPV/r-based regimen in treatment-naïve patients with human immunodeficiency virus type 1 (HIV-1) infection. This study aims to assess the efficacy and adverse effect events of LPV/r plus lamivudine and tenofovir or zidovudine as an initial antiretroviral treatment in HIV-1-infected individuals for whom cannot take efavirenz (EFV) or is allergic to EFV.

Methods: We performed a retrospective study of patients registering with the China's National Free Antiretroviral Treatment Program from July 2012 to January 2017, followed at a tertiary care hospital in Beijing, China. The primary outcome was the proportion of subjects with HIV-1 RNA ≤40 copies/ml at 6 and 24 months of treatment. We assessed the immunological response and adverse events.

Results: In total, 4,862 patients were enrolled in the study and 237 were eligible for analysis in each study arm. During the first six months, virological suppression was better with the LPV/r-based regimen than with the EFV-based regimen (93.80 vs 87.80% for $P < 0.05$). Viral suppression rates continued to increase until 12 months, remain steady thereafter until 24 months, for both groups. The multilevel analysis revealed that patients in the LPV/r group were more likely to display improvements in CD4 T-cell count over time than those in the EFV group ($P < 0.001$). Grade 3 or 4 laboratory adverse events were observed in 14 patients (5.91%) from the LPV/r group and three patients (1.20%) in EFV group.

Conclusion: Our findings demonstrate that LPV/r-containing regimens are effective and well-tolerated in Chinese treatment-naïve patients with HIV-1 infection.

Keywords: human immunodeficiency virus, first-line therapy, antiretroviral therapy, lopinavir/ritonavir, efavirenz, adverse effects

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Yonggang Zhang,
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Reviewed by:
David Katzenstein,
Stanford University, United States
Muhammad Usman,
University of Veterinary and Animal Sciences, Pakistan

***Correspondence:**
Lili Dai
lilydaier@163.com
Lijun Sun
sunlijunkity@163.com

†These authors have contributed equally to this work

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INTRODUCTION

Left untreated, human immunodeficiency virus (HIV) infection leads to a severe, life-threatening immunodeficiency syndrome. Worldwide, 36.9 million people were estimated to be living with HIV at the end of 2017 (World Health Organization Global Health Observatory (GHO), 2018; UNAIDS, 2018). The incidence of HIV infection has remained stable since 2005, but the number of people living with HIV (i.e. the prevalence of HIV infection) is steadily increasing (GBD 2015 HIV Collaborators, 2016). HIV prevalence is low in China, but this country is nevertheless ranked seventh worldwide in terms of the total number of infections, with 849,602 HIV-infected individuals registered by the end of September, 2018 (NCAIDS, NCSTD, China CDC, 2018), and this number probably remains underestimated due to inadequate surveillance and under reporting in low-income regions.

Antiretroviral therapy (ART) reduces the risk of disease progression and prevents HIV-1 transmission. Most ART guidelines worldwide recommend its use for all patients with HIV-1 infection, regardless of their CD4 T-cell counts. The China's National Free Antiretroviral Treatment Program (NFATP) was set up in 2003 and has significantly expanded access to ART in China. Morbidity and mortality in HIV-infected patients have decreased markedly in the last few years (AIDS and Hepatitis C Professional Group, 2018; Liu et al., 2018), but the availability of antiretroviral regimens remains limited in China, as in many developing countries.

According to the NFATP guidelines, the first-line regimen the treatment of HIV infection should consist of a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI): lamivudine (3TC), zidovudine (AZT) or tenofovir disproxil fumarate (TDF), and efavirenz (EFV) or nevirapine (NVP) or rilpivirine (RPV) (AIDS Professional Group, 2015). The protease inhibitor lopinavir/ritonavir (LPV/r)-based regimen is usually given as the second-line treatment when patients switch from the first-line regimen because of adverse events or drug resistance. Currently, the majority of HIV-1-infected patients are treated with a panel of free limited drugs provided by the Chinese government. No single tablet regimen such as EFV/3TC/TDF was available in China. However, LPV/r can be administered to treatment-naïve patients under some circumstances, such as in situations in which patients have low $CD4^+$ T-cell counts, drug resistance is a concern or testing is not available, the patient is female and wishes to have childbearing demand, the patient has a history of mental illness and cannot take EFV or the patient is allergic to EFV.

EFV and LPV/r are no longer recommended as first-line treatments in most developed countries, because of their adverse effects (Gunthard et al., 2014), but they are still widely used in underdeveloped and developing countries (World Health Organization, 2014). In China, 471,140 patients were registered as receiving ART under the NFATP by the end of 2015 (Liu et al., 2018). TDF, AZT, 3TC, EFV, and LPV/r are the most important antiretroviral drugs in clinical use, especially for LPV/r which is the main PI included in the NFATP (AIDS Professional Group, 2015; AIDS and Hepatitis C Professional Group, 2018). However, the previous study has shown that 22% patients displayed EFV concentrations out of the therapeutic range of 1–4 μg/ml in Chinese patients (13.1% < 1 μg/ml, 9.3% > 4 μg/ml) (Meng et al., 2015). These results show poor adherence with EFV in some patients and for others with excess of EFV, adverse events may occur. Thus, more clinical trials of new combinations need to be tested for new first-line ART regimens to improve adherence and tolerance.

Previous studies have suggested that LPV/r performs well in treatment-naïve HIV-1-infected individuals (Cohan et al., 2015; Ghosh et al., 2016; Jespersen et al., 2018), but little is known about the performance of LPV/r-based regimens in treatment-naïve patients in China. The aim of this study was, therefore, to compare the efficacy and adverse effects of LPV/r plus two NRTIs with those of EFV plus two NRTIs as a first-line ART in HIV-1-infected patients from a tertiary care hospital in Beijing, China.

MATERIALS AND METHODS

Study Population

We performed a follow-up study of patients who registered with the NFATP from July 2012 to January 2017 and were followed at the Center for Infectious Diseases of Beijing Youan Hospital, Capital Medical University in Beijing, China. This center is one of the most important HIV/AIDS health care centers participating in the NFATP in China, where more than 8,000 HIV-infected patients on ART are followed regularly.

The inclusion criteria were: 1) HIV-1 infection confirmed by western blotting; 2) patient >18 years of age. The exclusion criteria were: 1) pregnancy; 2) use of dual therapy; 3) history of ART before LPV/r therapy; 4) treatment for <3 months; 5) missing baseline data (at least one $CD4^+$ T-cell count or plasma HIV viral load at baseline missing); 6) patients who switched regimens. In total, 319 patients were using LPV/r as a first-line regimen and 2,832 patients were using EFV as a first-line regimen. We performed case-control matching to identify the best-matched pairs (1:1). Finally, 237 patients were included in each of the treatment arms in this study (**Figure 1**).

Ethics Statement

All the participants provided written informed consent for participation in the study and for the storage and use of their clinical samples for research. This study and other related experiments were approved by the Beijing Youan Hospital Research Ethics Committee, and written informed consent was obtained in accordance with the Declaration of Helsinki. The study was carried out in accordance with approved guidelines and regulations.

Data Collection

Baseline data were collected at treatment initiation. Follow-up visits were scheduled at 2 weeks, 1, 2, and 3 months, and then

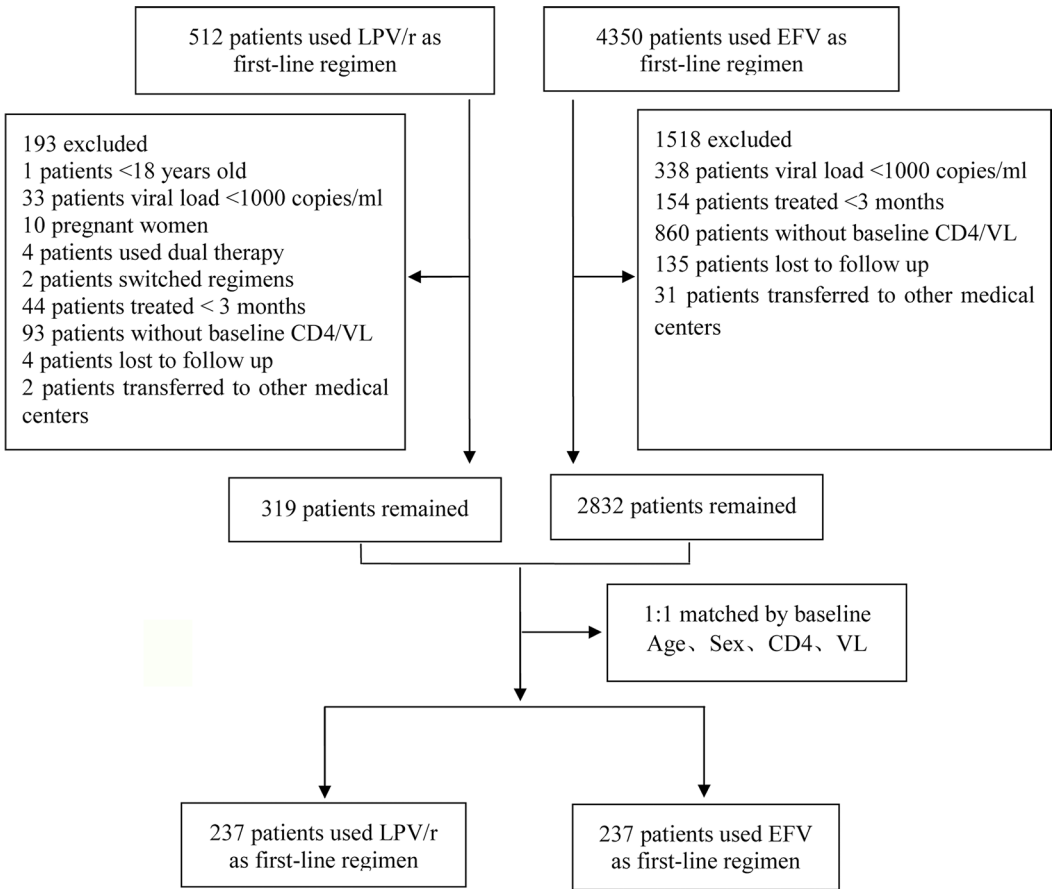

FIGURE 1 | Flowchart for patient selection.

every three months thereafter. The treatment groups were: 1) LPV/r-based regimen: LPV/r plus TDF/AZT plus 3TC; or 2) EFV-based regimen: EFV plus TDF/AZT plus 3TC. General data (demographic characteristics and symptoms) were collected at baseline. Leukocyte count, hemoglobin level, platelet count, alanine aminotransferase (ALT), total bilirubin, lipid, and glucose levels were determined at baseline and at each visit.

CD4⁺ T-Cell Count and Viral Load Measurement

CD4⁺ T-cell count and plasma HIV RNA levels were determined at baseline and every six months thereafter. Routine blood CD4⁺ T-cell counts (cells/μl) were measured by four-color flow cytometry with human monoclonal anti-CD4-APC, anti-CD3-FITC, anti-CD8-PE, and anti-CD45-PerCP antibodies (BD Multitest™, catalog No. 340499) on peripheral whole-blood samples from each patient according to the manufacturer's instructions. The cells were analyzed on a BD FACS Canto™ II flow cytometry system (BD Biosciences, San Jose, CA). HIV-1 viral load was determined with an automated real-time PCR-based *m*2000 system (Abbott Molecular Inc, Des Plaines, IL) in accordance with the manufacturer's instructions with a limit of detection of 40 copies/ml.

Observation Endpoint

The primary outcome was the proportion of subjects with HIV-1 RNA ≤40 copies/ml at 6, 12, 18 and 24 months. The main secondary endpoint was percentage change in CD4⁺ T-cell count from baseline at 6, 12, 18, and 24 months. Adverse events were assessed by determining: 1) the number of patients who discontinued or switched the ART regimen due to adverse events 2). the number of patients with laboratory abnormalities at least grade 3 due to drugs related. The severity of drug toxicity was evaluated according to the AIDS Clinical Trial Group toxicity grading scale.

Statistical Analysis

Variables that did not follow a normal distribution are presented as the median and interquartile range (IQR) and were analyzed in Wilcoxon rank sum tests. $P < 0.05$ was used to characterize the statistical significance. Categorical variables as age, sex, CD4⁺ T-cell count, HIV viral load, ART regimens, and laboratory values are presented as numbers and percentages and were analyzed in chi-squared tests. We used linear multilevel models to calculate differences in the change in CD4⁺ T-cell count from baseline to 24 months. Data were managed and analyzed with SAS version 9.14 (SAS Institute, Cary, North Carolina). Differences were considered statistically significant if $P < 0.05$ in two-tailed tests.

TABLE 1 | Baseline demographic and clinical characteristics.

Variables	LPV/r-based regimen (n = 237)	EFV-based regimen (n = 237)	*P*-value
Ages (years)			0.859
< 30	69	64	
30-40	124	126	
≥40	44	47	
Sex			
Men	227 (95.78%)	227 (95.78%)	1.000
Women	10 (4.22%)	10 (4.22%)	
Baseline CD4 (cells/μl)	273.00 (189.00-382.19)	281.50 (161.50-407.50)	0.976
$CD4^+$ T-cell count			0.122
≤100	40	27	
> 100 to ≤200	41	43	
> 200 to <350	75	94	
> 350 to <500	45	49	
≥500	36	24	
HIV RNA (log_{10} copies/ml)	4.25 (3.83-4.77)	4.26 (3.83-4.76)	0.942
HIV RNA (log_{10} copies/ml)			1.000
< 100 000	203	203	
≥100 000	34	34	
ART regimen			
TDF+3TC	201	204	0.696
AZT+3TC	36	33	
TC	3.86 (3.42-4.33)	3.89 (3.31-4.36)	0.632
TG	1.06 (0.82-1.47)	1.02 (0.78-1.45)	0.427
HDL-c	0.97 (0.83-1.14)	0.98 (0.85-1.12)	0.847
LDL-c	2.23 (1.92-2.67)	2.03 (1.92-2.67)	<0.001

RESULTS

Characteristics of the Patients

In total, 4,862 patients were included in the study: 237 patients were eligible for analysis in each arm of the study (**Figure 1**). The two groups were comparable at baseline in terms of age, sex, $CD4^+$ T-cell count, viral load, and serum lipid concentrations, but LDL-c concentration was higher in the group of patients on the LPV/r-based regimen [2.23 (1.92–2.67) vs. 2.03 (1.92–2.67); $P < 0.001$] (**Table 1**).

Virological Assessment

During the first six months, virological suppression was better in the LPV/r group than in the EFV group (93.80 vs. 87.80% and $P < 0.05$). Virological suppression rates continued to increase until 12 months, remaining stable thereafter until 24 months in both groups (**Figure 2**).

Immunological Response

Mean $CD4^+$ T-cell counts increased by 579.21 and 531.88 cells/μl between baseline and 24 months in the LPV/r and EFV groups, respectively. The multilevel analysis revealed that the patients in the LPV/r group were more likely to display an improvement in $CD4^+$ T-cell count over time than those in the EFV group ($P < 0.001$) (**Figure 3**).

Adverse Effects

None of the patients discontinued treatment due to adverse events. Adverse laboratory events of grade 3 or 4 were noted in 14 patients (5.91%) in the LPV/r group and three patients (1.20%) in the EFV group (**Table 2**).

DISCUSSION

Few data are available for the performance of first-line LPV/r-based regimens in treatment-naïve patients with HIV-1 infection (Cohan et al., 2015; Jespersen et al., 2018). This study therefore aimed to assess the efficacy and adverse effects of LPV/r plus 3TC

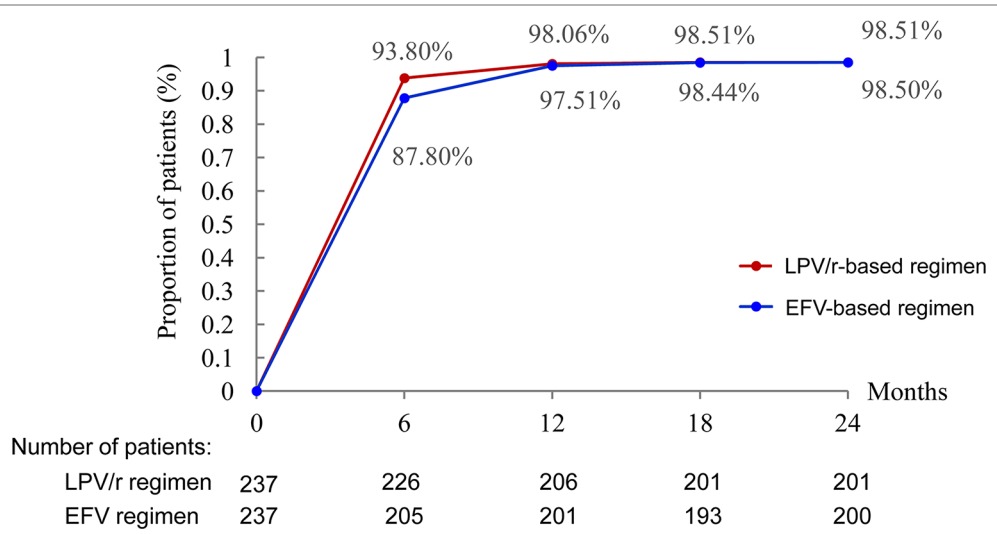

FIGURE 2 | Proportion of patients with HIV RNA <40 copies/ml. *$P < 0.05$, the difference in the proportion of patients with HIV RNA <40 copies/ml was significant in χ^2 tests.

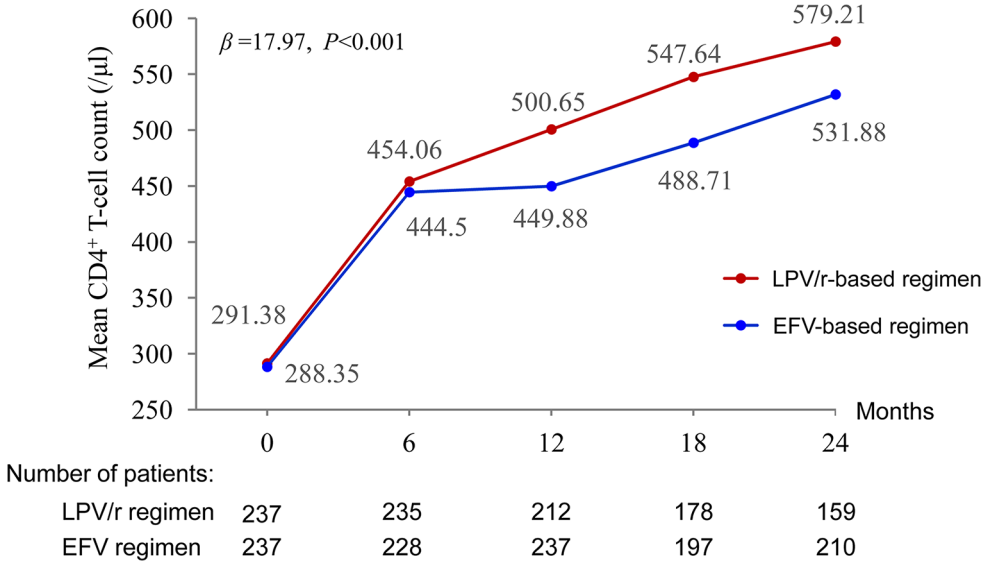

FIGURE 3 | Mean changes in the CD4+ T-cell counts of patients.

TABLE 2 | Laboratory abnormalities at 6, 12, 18, 24 months.

	LPV/r-based regimen (n = 237)	EFV-based regimen (n = 237)
Grade 3 or 4 laboratory abnormalities		
Leukocytes	0	0
Hemoglobin	0	0
Platelets count	0	0
Alanine aminotransferase (ALT)	0	0
Fasting glucose	2	0
Creatinine (CR)	0	0
Total cholesterol	2	1
Triglycerides	9	2
HDL cholesterol	0	0
LDL cholesterol	1	0

and TDF, or AZT as a first-line antiretroviral therapy in HIV-1-infected individuals, by comparison with a standard EFV-based regimen. The results obtained suggest that LPV/r- based ART has a good efficacy and adverse event profile for the Chinese treatment naïve patients with HIV-1 infection.

ART greatly improves the prognosis of HIV-infected patients, but factors such as adverse drug reactions, inadequate compliance, and drug resistance increase the likelihood of clinical and virological failure (Ghosn et al., 2018; Prabhu et al., 2019). LPV/r still plays a key role in treatment in developing countries, despite being an old drug that is no longer recommended for first-line treatment in Western countries, in which it is more widely used as a second-line therapy (Developed by the DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents, 2019). In areas with limited resources, such as China, LPV/r-based regimens are free and are the only option in situations in which EFV cannot be used due to primary drug resistance, allergies, hepatotoxicity, neurotoxicity, or pregnancy plans, for example. It thus remains a key drug in China's current anti-HIV treatment program.

Patients who agreed to ART with a LPV/r-based regimens displayed high rates of virological suppression and good immunological recovery. A study in pregnant women showed that first-line EFV- and LPV/r-based regimens both led to high levels of virological suppression and a low risk of transmission to the infant (Cohan et al., 2015). An African study showed that first-line LPV/r-based regimens triggered lower rates of treatment resistance than NNRTIs, but were not superior in terms of efficacy or severe adverse events (Jespersen et al., 2018). However, six studies have described patients who received EFV-containing regimens presented poor adherence due to neuropsychiatric adverse such as body heat, delusions, dizziness, anxiety, intense, and nightmares (Li et al., 2017), which can be associated with NNRTI resistance.

In our patients, virological suppression was achieved within the first six months in 93.8% of the patients who received initial LPV/r-based regimens. Viral suppression took longer to achieve in patients with baseline viral loads >100,000 copies/ml, consistent with the findings of previous study (Haile et al., 2016). Regardless of the stratification method used, the initial treatment of patients with LPV/r-based regimens resulted in complete virological suppression within 18 months. Some previous studies using a cutoff value of 40 copies/ml to define virological suppression have reported the achievement of virological suppression in 70% of patients on LPV/r-based regimens (Antiretroviral Therapy Cohort Collaboration, 2017).

In addition, another randomized controlled trial (ACTG) in Africa and Asia showed that >80% of the patients had suppressed plasma HIV RNA levels from week 12 onward (< 400 copies/ml) when treated with the LPV/r monotherapy (Kumarasamy et al., 2015). The greater benefits of a rapid decrease in plasma

HIV RNA levels, such as the prevention of HIV infection in HIV-negative individuals at high risk of exposure have been demonstrated in increasing numbers of studies. The prevalence of resistance to NNRTIs among previously untreated individuals living in areas of limited resources, such as Sub-Saharan Africa (5% resistance rate) (Gupta et al., 2012) and South Korea (2.7% resistance) (Park et al., 2016), has increased, and PI-based regimens may be more appropriate in these areas. The widespread use PI-based first-line therapies in resource-limited settings is not currently recommended, due to the high risk of resistance-related failure for second-line NNRTI/NRTI regimens (Hill et al., 2013), but Hill *et al.* suggested that LPV/r-based regimens might be superior to NNRTIs as an initial treatment, particularly in limited-resource settings, in which there may be resistance, but no access to drug resistance testing (Hill et al., 2013).

In this study, mean $CD4^+$ T-cell count had increased by 209.3 and 287.8 cells/μl relative to baseline at 12 and 24 months, respectively. Baseline $CD4^+$ T-cell counts are associated with immune response after ART. Patients with a $CD4^+$ T-cell count <200 cells/μl at the start of treatment had poorer immunologic outcomes than patients with >200 cells/μl, consistent with the findings of previous studies (Garcia et al., 2004). In this study, most patients had high $CD4^+$ T-cell counts at baseline, higher than those for patients on second-line ART in other studies (Luz et al., 2015), but similar to those reported in another study assessing LPV/r-based second-line ART (Patel et al., 2013). In an Iranian study, the authors reported that mean $CD4^+$ T-cell counts had increased by 139 cells/μl relative to baseline at 12 months (Rasooli-Nejad et al., 2017). The authors of a Ugandan study reported an increase in mean $CD4^+$ T-cell counts of 153 cells/μl at 12 months (Laker et al., 2014). We therefore hypothesized that initial treatment with LPV/r-based regimens might lead to a better immune response than switching to LPV/r-based regimens after first-line ART failure. Further studies are required to test this hypothesis.

Some adverse events were observed in our study population, but tolerance was good in most patients, and none of the patients discontinued treatment due to adverse events. An increase in LDL-c levels is one of the most important risk factors for atherosclerotic cardiovascular disease (ASCVD) (Stone et al., 2014a; Stone et al., 2014b; Catapano et al., 2017). Reducing LDL-c levels also reduces the risk of ASCVD and death (Baigent et al., 2005). We show here that median LDL-c levels had not increased after 24 months of ART. Similarly, HDL-c and TC levels remained good after 24 months (**Table 1**). In other studies, LPV/r-based regimens have generally been reported to be well-tolerated in terms of changes in lipid levels (Molina et al., 2007; Gathe et al., 2009), suggesting a limited impact of LPV/r-based regimens on cardiovascular risk. The results of this study suggest that the lipid profiles of patients taking AZT were generally poorer than those of patients taking TDF, especially for TC and LDL-c, but these differences were not significant, due to the small number of cases. A similar trend has been reported in previous studies (Feeney and Mallon, 2011; Souza et al., 2013; da Cunha et al., 2015; Ombeni and Kamuhabwa, 2016). No serious adverse events or severe hepatic dysfunction associated with LPV/r-based ART were observed. No patient discontinued ART or switched regimens because of adverse events.

However, some limitations of this study deserve mention. The sample size was small and all the patients came from a single center, limiting the extent to which the results can be generalized in whole China. No control group receiving a NNRTI was included. In addition, for reasons that were not always recorded on the patients' medical charts, only 159 patients were still in follow-up at 24 months, corresponding to an attrition rate of 33%. In addition, the higher loss of follow-up in the group of LPV/r may be related to the bi-daily administration, and/or to the digestive tract side effects. The data were limited to those available from the medical charts, and it was not possible to test additional biomarkers or factors associated with treatment failure. Additional studies are required to address these issues.

In conclusion, LPV/r-based ART was found to be beneficial and well-tolerated as a first-line ART in a resource-limited setting such as China. We observed high rates of virological suppression, immunological responses, and tolerability in treatment-naïve patients. Long-term (24 months) treatments with TDF+3TC+LPV/r or AZT+3TC+LPV/r were similarly beneficial. The use of LPV/r-based ART for treatment-naïve patients could be beneficial for patients for whom drug resistance testing is not available.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Beijing Youan Hospital Research Ethics Committee, Beijing Youan Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BS, YW, LD, and LS conceived the study, designed the experiments, and analyzed the data. TJ, HZ, ZL, AL, YS, and WH performed the experiments, carried out the data collection and data analysis. RZ, TZ, HW, SH, and LS contributed to reagents and materials. BS, YW, and LD wrote the article and revised the manuscript. All authors read and approved the final manuscript.

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REFERENCES

AIDS and Hepatitis C Professional Group, Society of Infectious Diseases, Chinese Medical Association, Chinese Center for Disease Control and Prevention. (2018). Chinese guidelines for diagnosis and treatment of HIV/AIDS (2018). *Chin. J. Intern. Med.* 57, 1–18. doi: 10.3760/cma.j.issn.0578-1426.2018.12.002

AIDS Professional Group, Society of Infectious Diseases, Chinese Medical Association (2015). Third edition of the guidelines for diagnosis and treatment of HIV/AIDS. *Chin. J. Clin. Infect. Dis.* 08 (05), 385–401. doi: 10.3760/cma.j.issn.1674-2397.2015.05.001

Antiretroviral Therapy Cohort Collaboration. (2017). Survival of HIV-positive patients starting antiretroviral therapy between 1996 and 2013: a collaborative analysis of cohort studies. *Lancet HIV* 4, e349–e356. doi: 10.1016/S2352-3018(17)30066-8

Baigent, C., Keech, A., Kearney, P. M., Blackwell, L., Buck, G., Pollicino, C., et al. (2005). Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 366, 1267–1278. doi: 10.1016/S0140-6736(05)67394-1

Catapano, A. L., Graham, I., De Backer, G., Wiklund, O., Chapman, M. J., Drexel, H., et al. (2017). 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *Rev. Esp. Cardiol. (Engl Ed)* 115. doi: 10.1016/j.rec.2017.01.002

Cohan, D., Natureeba, P., Koss, C. A., Plenty, A., Luwedde, F., Mwesigwa, J., et al. (2015). Efficacy and safety of lopinavir/ritonavir versus efavirenz-based antiretroviral therapy in HIV-infected pregnant Ugandan women. *AIDS* 29, 183–191. doi: 10.1097/QAD.0000000000000531

da Cunha, J., Maselli, L. M., Stern, A. C., Spada, C., and Bydlowski, S. P. (2015). Impact of antiretroviral therapy on lipid metabolism of human immunodeficiency virus-infected patients: old and new drugs. *World J. Virol.* 4, 56–77. doi: 10.5501/wjv.v4.i2.56

Developed by the DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents. (2019). Guidelines for the use of antiretroviral agents in adults and adolescents with HIV. https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf

Feeney, E. R., and Mallon, P. W. (2011). HIV and HAART-Associated Dyslipidemia. *Open Cardiovasc. Med. J.* 5, 49–63. doi: 10.2174/1874192401105010049

Garcia, F., de Lazzari, E., Plana, M., Castro, P., Mestre, G., Nomdedeu, M., et al. (2004). Long-term CD4+ T-cell response to highly active antiretroviral therapy according to baseline CD4+ T-cell count. *J. Acquir. Immune Defic. Syndr.* 36, 702–713. doi: 10.1097/00126334-200406010-00007

Gathe, J., da Silva, B. A., Cohen, D. E., Loutfy, M. R., Podzamczer, D., Rubio, R., et al. (2009). A once-daily lopinavir/ritonavir-based regimen is noninferior to twice-daily dosing and results in similar safety and tolerability in antiretroviral-naive subjects through 48 weeks. *J. Acquir. Immune Defic. Syndr.* 50, 474–481. doi: 10.1097/QAI.0b013e31819c2937

GBD 2015 HIV Collaborators. (2016). Estimates of global, regional, and national incidence, prevalence, and mortality of HIV, 1980-2015: the Global Burden of Disease Study 2015. *Lancet HIV* 3, e361–e387. doi: 10.1016/S2352-3018(16)30087-X

Ghosh, A. K., Osswald, H. L., and Prato, G. (2016). Recent Progress in the Development of HIV-1 protease inhibitors for the treatment of HIV/AIDS. *J. Med. Chem.* 59, 5172–5208. doi: 10.1021/acs.jmedchem.5b01697

Ghosn, J., Taiwo, B., Seedat, S., Autran, B., and Katlama, C. (2018). HIV. *Lancet* 392, 685–697. doi: 10.1016/S0140-6736(18)31311-4

Gunthard, H. F., Aberg, J. A., Eron, J. J., Hoy, J. F., Telenti, A., Benson, C. A., et al. (2014). Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society-USA Panel. *JAMA* 312, 410–425. doi: 10.1001/jama.2014.8722

Gupta, R. K., Jordan, M. R., Sultan, B. J., Hill, A., Davis, D. H., Gregson, J., et al. (2012). Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet* 380, 1250–1258. doi: 10.1016/S0140-6736(12)61038-1

Haile, D., Takele, A., Gashaw, K., Demelash, H., and Nigatu, D. (2016). Predictors of treatment failure among adult antiretroviral treatment (art) clients in bale zone hospitals, South Eastern Ethiopia. *PloS One* 11, e0164299. doi: 10.1371/journal.pone.0164299

Hill, A., McBride, A., Sawyer, A. W., Clumeck, N., and Gupta, R. K. (2013). Resistance at virological failure using boosted protease inhibitors versus nonnucleoside reverse transcriptase inhibitors as first-line antiretroviral therapy–implications for sustained efficacy of ART in resource-limited settings. *J. Infect. Dis.* 207 (Suppl 2), S78–S84. doi: 10.1093/infdis/jit112

Jespersen, S., Honge, B. L., Krarup, H., Medstrand, P., Sorensen, A., Medina, C., et al. (2018). Protease inhibitors or NNRTIs as first-line HIV-1 Treatment in West Africa (PIONA): a randomized controlled trial. *J. Acquir. Immune Defic. Syndr.* 79, 386–393. doi: 10.1097/QAI.0000000000001820

Kumarasamy, N., Aga, E., Ribaudo, H. J., Wallis, C. L., Katzenstein, D. A., Stevens, W. S., et al. (2015). Lopinavir/Ritonavir monotherapy as second-line antiretroviral treatment in resource-limited settings: week 104 analysis of AIDS clinical trials group (ACTG) A5230. *Clin. Infect. Dis.* 60, 1552–1558. doi: 10.1093/cid/civ109

Laker, E., Mambule, I., Nalwanga, D., Musaazi, J., Kiragga, A., and Parkes-Ratanshi, R. (2014). Boosted lopinavir vs boosted atazanavir in patients failing a NNRTI first line regimen in an urban clinic in Kampala. *J. Int. AIDS Soc.* 17, 19792. doi: 10.7448/IAS.17.4.19792

Li, H., Marley, G., Ma, W., Wei, C., Lackey, M., Ma, Q., et al. (2017). The role of ARV associated adverse drug reactions in influencing adherence among HIV-infected individuals: a systematic review and qualitative meta-synthesis. *AIDS Behav.* 21, 341–351. doi: 10.1007/s10461-016-1545-0

Liu, P., Tang, Z., Lan, G., Zhu, Q., Chen, H., You, Y., et al. (2018). Early antiretroviral therapy on reducing HIV transmission in China: strengths, weaknesses and next focus of the program. *Sci. Rep.* 8, 3431. doi: 10.1038/s41598-018-21791-2

Luz, P. M., Belaunzaran-Zamudio, P. F., Crabtree-Ramirez, B., Caro-Vega, Y., Hoces, D., Rebeiro, P. F., et al. (2015). CD4 response up to 5 years after combination antiretroviral therapy in human immunodeficiency virus-infected patients in Latin America and the Caribbean. *Open Forum Infect. Dis.* 2, ofv079. doi: 10.1093/ofid/ofv079

Meng, X., Yin, K., Wang, J., Dong, P., Liu, L., Shen, Y., et al. (2015). Effect of CYP2B6 gene polymorphisms on efavirenz plasma concentrations in chinese patients with HIV Infection. *PloS One* 10, e0130583. doi: 10.1371/journal.pone.0130583

Molina, J. M., Podsadecki, T. J., Johnson, M. A., Wilkin, A., Domingo, P., Myers, R., et al. (2007). A lopinavir/ritonavir-based once-daily regimen results in better compliance and is non-inferior to a twice-daily regimen through 96 weeks. *AIDS Res. Hum. Retroviruses* 23, 1505–1514. doi: 10.1089/aid.2007.0107

NCAIDS, NCSTD, CDC C. (2018). Update on the AIDS/STD epidemic in China the third quarter of 2018. *Chin. J. AIDS STD* 24, 1075. doi: 10.13419/j.cnki.aids.2018.11.01

Ombeni, W., and Kamuhabwa, A. R. (2016). Lipid profile in HIV-infected patients using first-line antiretroviral drugs. *J. Int. Assoc. Provid. AIDS Care* 15, 164–171. doi: 10.1177/2325957415614642

Park, M., Kee, M. K., Rhee, J., Park, J. Y., Kim, S. S., Kang, C., et al. (2016). The trend of transmitted drug resistance in newly diagnosed antiretroviral-naive HIV/AIDS patients during 1999-2012 in South Korea. *J. Clin. Virol.* 81, 53–57. doi: 10.1016/j.jcv.2016.06.001

Patel, D., Desai, M., Shah, A. N., and Dikshit, R. K. (2013). Early outcome of second line antiretroviral therapy in treatment-experienced human immunodeficiency virus positive patients. *Perspect. Clin. Res.* 4, 215–220. doi: 10.4103/2229-3485.120170

Prabhu, S., Harwell, J. I., and Kumarasamy, N. (2019). Advanced HIV: diagnosis, treatment, and prevention. *Lancet HIV* 6, e540–e551. doi: 10.1016/S2352-3018(19)30189-4

Rasooli-Nejad, M., Khazaee-Pool, M., Abbasian, L., Bayat Jozani, Z., Ahsani-Nasab, S., Moradmand Badie, B., et al. (2017). Assessing the efficacy of second-line antiretroviral treatment for hiv patients failing first-line antiretroviral therapy in iran: a cohort study. *Acta Med. Iran* 55, 233–240.

Souza, S. J., Luzia, L. A., Santos, S. S., and Rondo, P. H. (2013). Lipid profile of HIV-infected patients in relation to antiretroviral therapy: a review. *Rev. Assoc. Med. Bras (1992)* 59, 186–198. doi: 10.1016/S2255-4823(13)70454-5

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Bairey Merz, C. N., Blum, C. B., Eckel, R. H., et al. (2014a). 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 129, S1–45. doi: 10.1161/01.cir.0000437738.63853.7a

Stone, N. J., Robinson, J. G., Lichtenstein, A. H., Bairey Merz, C. N., Blum, C. B., Eckel, R. H., et al. (2014b). 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol.* 63, 2889–2934. doi: 10.1016/j.jacc.2013.11.002

UNAIDS. (2018). Global HIV & AIDS statistics — 2018 fact sheet. *WORLD AIDS DAY 2018*.

World Health Organization. (2014). March 2014 supplement to the 2013 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach.

World Health Organization Global Health Observatory (GHO). (2018). data. http://www.who.int/gho/hiv/en/.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Comparative Efficacy of Chinese Herbal Injections for the Treatment of Herpangina: A Bayesian Network Meta-Analysis of Randomized Controlled Trials

Xiaojiao Duan[†], *Haojia Wang*[†], *Jiarui Wu*[*], *Wei Zhou*, *Kaihuan Wang* and *Xinkui Liu*

Department of Clinical Chinese Pharmacy, School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, China

Background: Considering the limitations of broad-spectrum antiviral drugs for the treatment of herpangina and the extensive exploration of Chinese herbal injections (CHIs), systematic evaluation of the efficacy of different CHIs in the treatment of herpangina is a key imperative. In this study, we performed a network meta-analysis to investigate the efficacy of CHIs, including Reduning injection (RDN), Shuanghuanglian injection (SHL), Tanreqing injection (TRQ), Xiyanping injection (XYP), and Yanhuning injection (YHN), in the treatment of herpangina.

Methods: A systematic literature review including studies published before December 17, 2018, was conducted in several databases. The quality of the included studies was assessed using the Cochrane risk of bias tool. Data were analyzed using STATA 13.0 and WinBUGS 1.4.3 software. Surface under the cumulative ranking curve (SUCRA) probability values were applied to rank the examined treatments. Clustering analysis was performed to compare the effects of CHIs between two different outcomes.

Results: A total of 72 eligible randomized controlled trials involving 8,592 patients and five CHIs were included. All patients were under the age of 15 years, and most were under 7 years. The results of the network meta-analysis showed that RDN, XYP, and YHN had significantly better treatment performance than ribavirin. SHL (OR: 0.18; 95% CI: 0.09–0.34) and TRQ (OR: 0.18; 95% CI: 0.10–0.31) were obviously superior to ribavirin with respect to total clinical effectiveness. The results of SUCRA and cluster analysis indicated that RDN is the best intervention with respect to total clinical effectiveness, antipyretic time, and blebs disappearing time. Fifty-four studies described adverse drug reactions/adverse drug events (ADRs/ADEs), and 32 studies reported ADRs/ADEs in detail.

Conclusions: CHIs were found to be superior to ribavirin in terms of treatment performance and may be beneficial for patients with herpangina. RDN had the potential to be the best CHI with respect to all outcome measures. More evidence is needed to assess the safety aspects of CHIs.

Keywords: network meta-analysis, Bayesian model, Chinese herbal injections, herpangina, ribavirin

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Yonggang Zhang,
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Priyia Pusparajah,
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University of Mississippi Medical Center, United States

****Correspondence:***
Jiarui Wu
exogamy@163.com

[†]*These authors have contributed equally to this work*

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INTRODUCTION

Herpangina is a common pediatric disease that is mainly caused by Coxsackie A virus; respiratory and fecal-oral routes are the main routes of transmission. Coxsackie A virus is a small RNA virus that is present in the intestines. The virus exhibits rapid transmission, especially in summer and early autumn. Children in the age group of 1–7 years are particularly vulnerable to infection (Jiang et al., 2015; Huang, 2016). Children infected with herpangina can manifest sore throat, excessive salivation, fever, oral herpes, anorexia, and other symptoms. Enteroviruses are also known to cause serious diseases such as myocardial damage or myocarditis (Wu, 2018; Guo and Li, 2019). Currently, there is no specific treatment for herpangina. Antiviral drugs, symptomatic supportive care, and prevention of complications are the mainstays of treatment (Guo and Li, 2019). Ribavirin is a broad-spectrum antiviral drug that is commonly used for the treatment of herpangina. However, the mechanism of action of ribavirin is highly dependent on viral adenosine kinase; this results in a high probability of the development of drug resistance, which in turn affects the therapeutic effect (Li and Zhan, 2017; Liu et al., 2019). Several recent studies have documented the efficacy of Chinese herbal injections (CHIs) in the treatment of herpangina (Zhu et al., 2014; Xu et al., 2016; Xia, 2016). However, several varieties of CHIs have been used to treat herpangina, and further research is required to identify the best type of CHI for this purpose. Therefore, in this study, we used the network meta-analysis (NMA) method to systematically evaluate the efficacy of different CHIs in the treatment of herpangina. The objective was to identify an optimal intervention measure and provide a basis for clinical drug use.

METHODS

This study is reported in strict accordance with the standard format of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Specification: PRISMA Extension Statement specification (Hutton et al., 2015; Ge et al., 2017).

Search Strategy

PubMed, the Cochrane Library, Embase, the Chinese Biological Medicine Literature Service System (SinoMed), the China National Knowledge Infrastructure (CNKI) database, the Chinese Scientific Journal Database (VIP), and the Wanfang Database were searched for randomized controlled trials (RCTs) of CHIs for the treatment of herpangina. Studies published as of December 17, 2018 were eligible for inclusion. In addition, the reference lists of the included studies were manually searched to identify relevant literature. There were three parts of the search strategy, including herpangina, Chinese herbal injection, and random controlled trial. A total of 132 types of CHIs incorporating national standards of the Chinese Food and Drug Administration and 36 kinds of Chinese medicine-derived chemical injections were included in the prescreening. The five CHIs that were finally included in the analysis were Reduning injection (RDN), Shuanghuanglian injection (SHL), Tanreqing injection (TRQ), Xiyanping injection (XYP), and Yanhuning injection (YHN). The detailed search strategy is described in **Presentation File**.

Inclusion Criteria

Types of Studies

RCTs of CHIs for the treatment of herpangina were eligible if they were referred to as "random," with or without blinding.

Types of Participants

All patients included were clinically diagnosed with herpangina according to clear diagnostic criteria, with no limitations of sex, race, or age.

Types of Interventions

The interventions included were comparisons between CHIs and ribavirin or between different types of CHIs. Ribavirin and CHIs were administered intravenously; in addition, according to the patient's condition, certain symptomatic supportive treatments were adopted (e.g., cooling, rehydration, maintenance of water and electrolyte balance, and antibiotic therapy for concurrent bacterial infection). No limitations were imposed with respect to the dosage or treatment course. No other Chinese medicine or remedies were used, such as decoction, proprietary Chinese medicine, acupuncture, or massage.

Types of Outcomes

Outcome indicators included total clinical effectiveness, antipyretic time, blebs disappearing time, and adverse reactions (ADRs)/adverse events (ADEs). Total clinical effectiveness = (total number of patients—;number of patients in whom treatment was ineffective)/total number of patients×100%. The evaluation criteria for efficacy were based on the posttreatment recovery of clinical symptoms and signs; ineffective treatment implies deterioration or no change in symptoms and signs after the treatment course.

Data Extraction and Quality Assessment

All retrieved studies were managed using NoteExpress software. After excluding duplicates, two researchers independently screened the retrieved studies based on the inclusion and exclusion criteria and extracted the data from the included RCTs. The titles and abstracts of retrieved studies were screened to exclude animal studies, literature reviews, and other unrelated articles. Subsequently, studies that met the inclusion criteria were identified, and their full texts were reviewed. A specially designed form (created using Microsoft Excel 2016 software) was used to extract data pertaining to the following information from the included studies: (1) name of first author and the year of publication; (2) basic characteristics of patients: the numbers of patients in the treatment group and the control group, sex distribution, average age or age range, interventions, and treatment details; (3) outcome measures;

TABLE 1 | Characteristics of the studies included in this meta-analysis.

Study ID	Random method	Cases (A/B/C)	Sex (M/F)	Age	Intervention A	Intervention B	Intervention C	Basic treatment	Course (d)	Consistent baseline	Outcomes	ADRs/ ADEs
Xie, 2017	Random	40/40	47/33	A:3–12(8.8 ± 1.1) B:3–12(8.8 ± 1.3)	RDN: (age) 3–5 < 10 ml; 6–10 = 10 ml; 11–12 = 15 ml	Ribavirin: 10 mg/(kg·d)	NA	NA	5–7	Y	①②③	NR
Xiao, 2016	Random	40/40	43/37	A:3–7 B:3–7	RDN: 0.5–0.8 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Rehydration; cooling	5	Y	①	NR
Feng et al., 2015	Random number table	45/45	51/39	A:0.6–7(3.8 ± 2.2) B:0.5–7(3.6 ± 2.3)	RDN: 0.6 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	NA	5	Y	①②③	N
Liu, 2015	Random	54/51	56/49	0.5–5(3.7 ± 2.2)	RDN: 0.5 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Symptomatic supportive treatment; bacterial infection combined with antibiotic treatment	3	Y	①②③	N
Wang and Li, 2015	Random	92/90	98/84	A:0.5–5(2.2 ± 1.5) B:0.7–4(2 ± 1.2)	RDN: 0.5 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic supportive treatment; bacterial infection combined with azithromycin or penicillin treatment	5–7	Y	①②	Detailed description
Deng and Tang, 2014	Random number table	90/90	102/78	0.5–7(3.12 ± 2.22)	RDN: 0.6–0.8 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Routine care, cooling, rehydration, maintenance of water and electrolyte balance and other symptomatic supportive treatment; bacterial infection combined with antibiotic treatment	5	Y	①②③	Detailed description
Dong, 2014	Random	40/40	45/35	A:0.6–7(3.1 ± 1.2) B:0.5–7(3.4 ± 1.3)	RDN: 0.6 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Routine care, cooling, rehydration, maintenance of water and electrolyte balance and other symptomatic supportive treatment; bacterial infection combined with antibiotic treatment	3–5	Y	①②③	Detailed description
Hu, 2014	Random	50/50	53/47	0.5–4	RDN: 0.5 ml/(kg·d)	Ribavirin: 10–15mg/(kg·d)	NA	Oral care; antipyretics; vitamin supplements; fluid replacement, etc.	5–7	Y	①②③	Detailed description
Ji et al., 2014	Random number table	95/95	103/87	0–14	RDN: ≤3 (age), 5ml; > 3, 10 ml	Ribavirin: 10–15 mg/(kg·d)	NA	Bacterial infection: plus antiinfection treatment with cephalosporins or penicillin antibiotics; mycoplasma infection: plus macrolide antiinfective treatment, the same symptomatic treatment in both groups	3–5	Y	①②③	NR
Ke, 2014	Random	37/31	37/31	A:1–7(3.5 ± 2.3) B:1–7(3.6 ± 2.1)	RDN: ≤3 (age), 5ml; > 3, 10 ml	Ribavirin: 10–15 mg/(kg·d)	NA	Symptomatic treatment	3	Y	①②③	Detailed description
Tan, 2014	Random number table	110/110	130/90	A:0.4–7(3.6 ± 2.5) B:0.4–7(3.9 ± 2.1)	RDN: 0.5–0.8 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Routine care, cooling, rehydration to maintain water and electrolyte balance and other symptomatic supportive treatment; bacterial infection plus oral antibiotics	5–7	Y	①②③	Detailed description
Yu and Qian, 2014	Random number table	60/60	76/44	A:0.4–7(3.6 ± 2.5) B:0.4–7(3.8 ± 2.2)	RDN: 0.5–0.8 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Routine care, cooling, rehydration to maintain water and electrolyte balance and other symptomatic supportive treatment; bacterial infection plus oral antibiotics	5	Y	①②③	N

(Continued)

TABLE 1 | Continued

Study ID	Random method	Cases (A/B/C)	Sex (M/F)	Age	Intervention A	Intervention B	Intervention C	Basic treatment	Course (d)	Consistent baseline	Outcomes	ADRs/ ADEs
Yang, 2013	Random	56/56	60/52	0.8–4	RDN: 0.5–0.7 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Routine symptomatic, supportive, antiinfective treatment	5–7	Y	①	N
Zhang, 2013	Random	23/19	25/17	0–14	RDN: ≤3 (age), 5ml; > 3, 10 ml	Ribavirin: 10–15 mg/(kg·d)	NA	Children with bacterial infection use antiinfective treatment with cephalosporin or penicillin, the same symptomatic treatment in both groups	3–5	Y	①②③	Detailed description
Chen, 2012	Random	54/54	NR	1–7	RDN: 0.5–0.8 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic, support, antiinfective treatment	3–5	Y	①②③	Detailed description
Pu, 2012	Random	50/50	53/47	0.5–6	RDN: 0.6 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Cooling; antiinfectives with azithromycin or penicillin	3–5	Y	①②③	Detailed description
Wang, 2012	Random	92/76	NR	NR	RDN: 0.6 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Routine care, cooling, rehydration to maintain the balance of water and electricity and other symptomatic supportive treatment; bacterial or mycoplasma infection plus related antibiotics	3–5	Y	①②③	N
Zhang et al., 2012	Random	96/96	111/81	A:0.8–12(5.2 ± 1.5) B:0.7–7(5.0 ± 1.7)	RDN: 0.5 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	NA	3	Y	①	Detailed description
Zhang, 2012	Random number table	100/100	113/87	1–7(3.23 ± 2.22)	RDN: 0.5–0.7 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Routine care, cooling, rehydration to maintain water and electrolyte balance and other symptomatic supportive treatment; bacterial infection plus antibiotic treatment	5	Y	①②③	N
Cai, 2011	Random	60/60	68/52	0.5–7(4.12 ± 3.22)	RDN: 0.6 ml/(kg·d)	Ribavirin: 10–15mg/(kg·d)	NA	Routine care, cooling, rehydration to maintain water and electrolyte balance and other symptomatic supportive treatment; bacterial infection plus antibiotic treatment	3–5	Y	①②③	N
Zeng, 2011	Random	50/50	58/42	1–14 (7.5)	RDN: 0.6 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	NA	5–7	Y	①	NR
Sun et al., 2011	Random	44/44	51/37	0.6–8(3.9 ± 3.2)	RDN: 0.5–0.8 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic supportive treatment; bacterial infection plus antibiotic treatment	5–7	Y	①②③	Detailed description
Xie, 2011	Random	45/45	48/42	0.5–6	RDN: 0.6 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Cooling; bacterial infections with azithromycin or penicillin against infection	3–5	Y	①②③	Detailed description
Guo, 2010	Random	60/60	62/58	0.5–5	RDN: < 2 (age) 0.5–0.8 ml/d	Ribavirin: 10–15mg/(kg·d)	NA	NA	5–7	Y	①	N
Xiao, 2010	Random	53/52	55/50	0.5–7	RDN: 0.5–0.8 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic supportive treatment; bacterial infection plus antibiotic treatment	5–7	Y	①②③	Detailed description
Xu et al., 2009	Random number table	60/60	64/56	1–7	RDN: 0.6–0.8 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	NA	3–5	Y	①②③	Detailed description

(Continued)

TABLE 1 | Continued

Study ID	Random method	Cases (A/B/C)	Sex (M/F)	Age	Intervention A	Intervention B	Intervention C	Basic treatment	Course (d)	Consistent baseline	Outcomes	ADRs/ ADEs
Pang et al., 2008	Random number table	42/42	53/31	1–7	RDN: 0.6–0.8 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Conventional fluid therapy and symptomatic treatment	3–5	Y	①②③	Detailed description
Wang, 2013	Random	60/60	64/56	0.42–5	SHL: 60 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Cooling; bacterial infection combined with antibiotic treatment	3–6	Y	①	NR
Zhao, 2012	Random	44/44	54/34	0.58–5	SHL: 60 mg/(kg·d)	Ribavirin	NA	Basic oral care; oral multivitamin B	3	NR	①	N
Peng and Tao, 2010	Random	66/40	63/43	0–14	SHL: 60 mg/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Oral care; bacterial infection combined with antibiotic treatment	3–7	Y	①	N
Cao, 2008	Random	40/36	46/30	0.67–5	SHL: 60 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Drink more water; supplement vitamin B, vitamin B family	7	Y	①②	Detailed description
Feng and Peng, 2013	Random	80/72	79/73	A: 0.92 ± 0.5 B: 1 ± 0.42	TRQ: 0.3–0.5 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Oral care, fluid replacement, symptomatic and other conventional comprehensive treatment	5–7	Y	①	N
Cai, 2012	Random	108/102	110/ 100	A: 0.42–5.5 B: 0.42–6	TRQ: 0.5 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Supplemented with intravenous infusion of water-soluble vitamins; correct water and electrolyte disorders according to the situation; infected patients were given intravenous infusion of cefotiam	5	Y	①	NR
Wen, 2012	Random	24/23	25/22	NR	TRQ: 0.3–0.5 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	NA	3–5	Y	①	N
Tan, 2011	Random	68/62	69/61	A:0.33–10(3.1 ± 2.6)B:0.42–11(2.8 ± 3.3)	TRQ: 0.5 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic support and other treatments; bacterial infections given antibiotic treatment	5	Y	①②	NR
Jiang, 2009	Random sampling	50/50	54/46	A: 1 ± 0.42 B: 1.1 ± 0.33	TRQ: 0.3–0.5 ml/(kg·d)	Ribavirin: 0.1 mg/(kg·d)	NA	NA	3	Y	①	NR
Xia, 2016	Random number table	46/40	44/42	1–5	XYP: 0.2 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Antipyretic	3	Y	①②③	N
Cao, 2015	Random	25/23	24/24	A:0.7–2.5 B:1–3.2	XYP: 0.2 ml/(kg·d)	Ribavirin: 10 ml/(kg·d)	NA	Antipyretic	5	Y	①	NR
Lin, 2014	Random	48/48	51/45	0.5–3	XYP: 5 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Dietary guidance; according to the nature and degree of dehydration, rehydration to correct water, electrolyte and acid-base balance disorders; antipyretic, symptomatic treatment	3	Y	①	N
Yang et al., 2013	Random	123/123	130/ 116	0.4–6	XYP: 0.2–0.4 ml/(kg·d)	Ribavirin: 15 mg/(kg·d)	NA	Symptomatic supportive treatment	3	Y	①	N
Zeng et al., 2013	Random	60/60	68/52	0.7–5	XYP: 5–10 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Cooling; treatment of bacterial infection with cefotaxime	5	Y	①②③	Detailed description
Wang, 2013	Random	45/45	49/41	A:0.7–7 B:0.7–6	XYP: 20 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	General care; symptomatic, supportive care; multivitamin supplementation	5–7	Y	①②③	NR
Zhou, 2013	Random	72/68	82/58	0.5–5	XYP: 0.2–0.4 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Symptomatic supportive treatment	5	Y	①②	Detailed description

(Continued)

TABLE 1 | Continued

Study ID	Random method	Cases (A/B/C)	Sex (M/F)	Age	Intervention A	Intervention B	Intervention C	Basic treatment	Course (d)	Consistent baseline	Outcomes	ADRs/ ADEs
Su and Ke, 2012	Random number table	195/194	202/ 187	1–7	XYP: 0.1–0.2 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Routine rehydration and symptomatic treatment, if the child's temperature is >38.50°C, use short-acting antipyretic agent as appropriate	3–5	Y	①②③	Detailed description
Jia and Tian, 2012	Random	70/50	76/44	0.5–5	XYP: 5 mg/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Symptomatic supportive treatment	5	Y	①	Detailed description
Li et al., 2011	Random	39/37	42/34	A:0.3–7 B:0.25–7	XYP: 0.2–0.4 ml/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Basic treatment of respiratory tract isolation, symptomatic treatment, supportive treatment, etc.	3	Y	②③	N
Yang, 2011	Random	31/30	NR	0.3–5	XYP: 10 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic treatment such as fever, vitamin B supplements and fluid replacement	5	Y	①	NR
Zhang, 2011	Random sampling	34/38	26/46	A:(1.3 ± 0.41) B: (1.5 ± 0.43)	XYP: 5–8 mg/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Children with moderate to high fever are given oral or intramuscular injection of antipyretics to cool down	3	Y	①②③	N
Zhang, 2011	Random	42/40	NR	0.3–5	XYP: 10 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic supportive treatment	5	Y	①②③	Detailed description
He and Peng, 2010	Random	42/38	45/35	A:0.5–4 B:0.5–5	XYP: 5 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Symptomatic supportive treatment	5	Y	①②	Detailed description
Shen, 2010	Random	25/25	27/23	A:0.3–3.5 B:0.42–4	XYP: 0.2–0.3 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Antipyretic; oral care; secondary bacterial infection plus penicillin or cephalosporin treatment	3	Y	①	N
Guo, 2009	Random	80/80	85/75	1–7	XYP: 5–10 mg/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Symptomatic supportive treatment	3–5	Y	①	Detailed description
Chen et al., 2008	Random number table	36/33	38/31	1–7	XYP: 0.2–0.4 ml/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Routine rehydration and symptomatic treatment, if the child's temperature is >38.50°C, use short-acting antipyretic agent as appropriate	3	Y	①②③	N
Huang et al., 2008	Random	68/62	76/54	A:0.5–4 B:0.5–5	XYP: 5 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Antipyretic; supplemented with vitamin B, vitamin B, ceftriaxone sodium or amoxicillin clavulanate potassium for antiinfective treatment	5	Y	①②	Detailed description
Qu et al., 2016	Random	40/40	45/35	0.5-5	YHN: 5–10 mg/(kg·d)	Ribavirin: 10-15mg/(kg·d)	NA	Give appropriate and supportive care as appropriate	3-5	Y	①	NR
Yang, 2014	Random number table	175/175	189/ 161	1-7	YHN: 5–10 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Give intravenous rehydration and symptomatic treatment, and give ibuprofen antipyretic as appropriate for body temperature >38.5°C	3-5	Y	①②③	Detailed description
Dong and Feng, 2013	Random	40/40	42/38	0.5–7	YHN: 5 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	High fever given antipyretics; rest; drinking more water; prevention of complications; antibiotics in patients with bacterial infections	5	Y	①	NR
Song and Fan, 2013	Random	40/36	49/27	0.5–2	YHN: 5–10 mg/(kg·d)	Ribavirin: 10–15mg/(kg·d)	NA	Rehydration and symptomatic treatment; bacterial infections treated with antibiotics	5–7	Y	①②③	NR

(Continued)

TABLE 1 | Continued

Study ID	Random method	Cases (A/B/C)	Sex (M/F)	Age	Intervention A	Intervention B	Intervention C	Basic treatment	Course (d)	Consistent baseline	Outcomes	ADRs/ ADEs
Li, 2012	Random	42/38	42/38	A:5.6(1–7) B:5.8(1–7)	YHN: 5–10 mg/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Oral care topical treatment	5	Y	①	Detailed description
Wang et al., 2012	Random	120/120	100/ 140	0–7	YHN: 3–8 mg/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	NA	3–5	Y	①	NR
Fang, 2011	Random	67/66	69/64	1–7	YHN: 5–10 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Conventional fluid replacement and symptomatic treatment; if the body temperature is >38.5°C, use a short-acting antipyretic agent as appropriate.	3	Y	①	N
Guo, 2011	Random	44/44	48/40	0.5/4	YHN: 5–10 mg/(kg·d)	Ribavirin: 10 mg/(kg·d)	NA	Antipyretic; supplemented with vitamin B, vitamin B2, ceftazidime, or cefuroxime for antiinfective treatment	5	Y	①③	Detailed description
Li et al., 2011	Random	40/40	45/35	0.5–5	YHN: 5–10 mg/(kg·d)	Ribavirin: 10–15mg/(kg·d)	NA	Give appropriate and supportive care as appropriate	3–5	Y	①	NR
Yin, 2011	Random	30/30	30/30	A:1–7 B:1.5–6.5	YHN: 5–10 mg/(kg·d)	Ribavirin: 10-15mg/(kg·d)	NA	Give appropriate and supportive care as appropriate	3–5	Y	①②③	NR
Lv, 2009	Random	30/18	28/20	A:0.5–4 B:0.5–3.5	YHN: 5–10 mg/(kg·d)	Ribavirin: 10–5mg/(kg·d)	NA	Pay attention to rest; drink plenty of water; add vitamin B, vitamin B; cool down	5	Y	①②	Detailed description
Hu, 2008	Random	30/30	28/32	0.7–4	YHN: 5–10 mg/(kg·d)	Ribavirin: 10–15mg/(kg·d)	NA	Give appropriate and supportive care as appropriate	4–7	Y	②③	N
Wei, 2007	Random	63/63	NR	1–7	YHN: 5–10 mg/(kg·d)	Ribavirin: 10–15 mg/(kg·d)	NA	Give appropriate and supportive care as appropriate	3	Y	②③	N
Guo et al., 2014	Random	38/35	38/35	A:2.38 ± 1.56 B:2.58 ± 1.54	YHN: 5–10 mg/ (kg·d)	RDN: 0.5–0.7 ml/(kg·d)	NA	Intravenous infusion of water-soluble vitamins; oral care; symptomatic treatment; hyperthermia preheat treatment; supplementation of liquids and electrolytes	5–7	Y	①②③	Detailed description
Zhu, 2013	Random	60/60	67/53	1–5	TRQ: 0.5–0.3 ml/(kg·d)	SHL: 60 mg/(kg·d)	NA	Rehydration and symptomatic treatment; infected with antibiotics	NR	Y	①②	NR
Wang, 2012	Random	40/40/40	NR	3.16 ± 2.22	XYP: 5 mg/(kg·d)	RDN: 0.5–0.8 ml/(kg·d)	YHN: 3–5 mg/(kg·d)	All patients were given routine support, cooling, rehydration to maintain water and electrolyte balance and other symptomatic supportive treatment; patients with concurrent bacterial infections were treated with antibiotics	5	Y	①②③	Detailed description

(Continued)

TABLE 1 | Continued

Study ID	Random method	Cases (A/B/C)	Sex (M/F)	Age	Intervention A	Intervention B	Intervention C	Basic treatment	Course (d)	Consistent baseline	Outcomes	ADRs/ ADEs
Zhou, 2012	Random	60/60	62/58	0.3–6	RDN: 0.6 ml/(kg·d)	YHN: 10 mg/(kg·d)	NA	Light diet; oral care; those with high fever to physical cooling and antipyretic cooling; those with vomiting and diarrhea to microecological regulators and intestinal mucosal protective agents, supplements with liquids and electrolytes; those with bacterial infections apply appropriate antibiotics, etc.	3–5	Y	①	Detailed description
Liu and Li, 2011	Random	90/90/90	147/ 123	A:.05–6.5 B:0.5–6.7 C:0.4–6.6	XYP: 5 mg/(kg·d)	Ribavirin: 10 mg/ (kg·d)	RDN: 0.5 ml/ (kg·d)	Symptomatic support treatment such as antipyretic and drinking water	7	Y	①②③	Detailed description

M, male; F, female; RDN, Reduning injection; SHL, Shuanghuanglian injection; TRQ, Tanreqing injection; XYP, Xiyanping injection; YHN, Yanhuning injection; NA, Not Applicable; NR, Not Reported; Y, Yes; N, No; ①: The Rate of Clinical Efficacy; ②: Antipyretic time; ③: Blebs disappearance time.

and (4) study types and main factors affecting the risk of bias. Any disagreement between two researchers during the screening of studies and extraction of data was resolved by consensus or by consulting a third researcher.

Two authors independently assessed the risk of bias in the included studies in accordance with the risk of bias assessment tool recommended in the Cochrane Handbook 5.1 (Higgins and Green, 2010). The following elements were assessed: (1) selection bias associated with random sequence generation; (2) selection bias associated with allocation concealment; (3) performance bias: blinding of participants and personnel; (4) detection bias: blinding of outcome assessment; (5) attrition bias: integrity of outcome data; (6) reporting bias: selective reporting; and (7) bias from other sources. Each element was categorized as "low risk," "high risk," or "unclear." "Low risk" implies that the implementation method is correct or does not affect the result; "high risk" implies that the implementation method is incorrect and affects the measurement of the result; "unclear" means that the information is insufficient, and the risk of bias cannot be judged. Consensus was attained by discussion or involving a third researcher.

Data Analysis

WinBUGS 1.4.3 software was used to perform NMA, and the Markov chain Monte Carlo method with random-effects model was performed for Bayesian inference. In the WinBUGS software, the number of iterations was set as 200,000, with the first 10,000 iterations used for burn-in to eliminate the impact of the initial value. On NMA, the odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for the binary outcomes; the mean difference (MD) and 95% CI were calculated for continuous outcomes. When the 95% CI for the OR value did not contain 1 and the 95% CI for MD value did not contain 0, the difference between groups was deemed to be statistically significant. Stata 13.0 software was used to map the network of different interventions for each outcome measure, showing the results of the direct and indirect comparison of CHIs. When using the results of WinBUGS software with Stata software, the surface under the cumulative ranking probability (SUCRA) of different CHIs in each outcome index was obtained. The larger the SUCRA and the higher the ranking, the greater the probability that the CHI is the best intervention. A comparison-adjusted funnel plot was used to assess potential publication bias. If points on both sides of the midline in the funnel diagram were symmetric, which meant the correction guideline was at right angles to the midline, it was considered indicative of no significant publication bias. The cluster analysis method was used to comprehensively analyze and compare interventions for two different outcome indicators; then, the optimal injection variety for the two outcome indicators was obtained. The farther from the origin in the cluster map, the better the effect is in these two outcome indicators. If there was a closed loop, the inconsistency test was used to evaluate the consistency of each closed loop, and the inconsistency factors (IFs) and 95% CI were calculated. When the 95% CI contained 0, the consistency was good; otherwise, the closed loop was considered to exhibit significant inconsistency.

RESULTS

Search Results

Out of the 1,123 retrieved articles, 72 RCTs (shown in **Table 1**) were selected and included in the NMA. Further details of the literature screening process are presented in **Figure 1**. Two studies were three-arm studies (RDN vs. XYP vs. YHN, and RDN vs. XYP vs. ribavirin), while all other studies were two-arm studies. Among these, 67 RCTs investigated CHIs vs. ribavirin as the intervention, including five kinds of CHIs: RDN (27 RCTs), SHL (4 RCTs), TRQ (5 RCTs), XYP (18 RCTs), and YHN (13 RCTs). The remaining three RCTs investigated CHI vs. another CHI as the intervention: RDN vs. YHN (2 RCTs) and TRQ vs. SHL (1 RCT). All included studies were published in Chinese, and the year of publication ranged from 2007 to 2018.

Inclusion Studies and Characteristics

The 72 RCTs included 8,592 patients; of these, 1,866 patients were treated with RDN, 270 patients received SHL, 390 patients received TRQ, 1,211 patients received XYP, 896 patients received YHN, and 3,959 patients received ribavirin. Six studies did not report the sex distribution in the study population; the remaining studies enrolled 4,320 male patients, which accounted for 54.50% (4,320/7,927). All included patients were under the age of 15 years, and most were under 7 years. The maximum sample size of the included RCTs was 195, and the minimum sample size was 18. Sixty-nine RCTs (95.83%, five CHIs) reported total clinical effectiveness, 45 RCTs (62.50%, five CHIs) reported antipyretic time, and 38 RCTs (52.78%, three CHIs) reported blebs disappearing time. The network graph of CHIs with different outcomes is shown in **Figure 2**. All treatment courses lasted < 7 days. The details of the included studies are shown in **Table 1**.

Methodological Quality

Of the 72 included studies, 12 RCTs used a random number table for group allocation, while two RCTs used a random sampling method. The selection bias associated with random sequence generation of the above studies was evaluated as "low risk." All studies reported complete data, and their attrition bias was evaluated as "low risk." One RCT did not indicate whether the

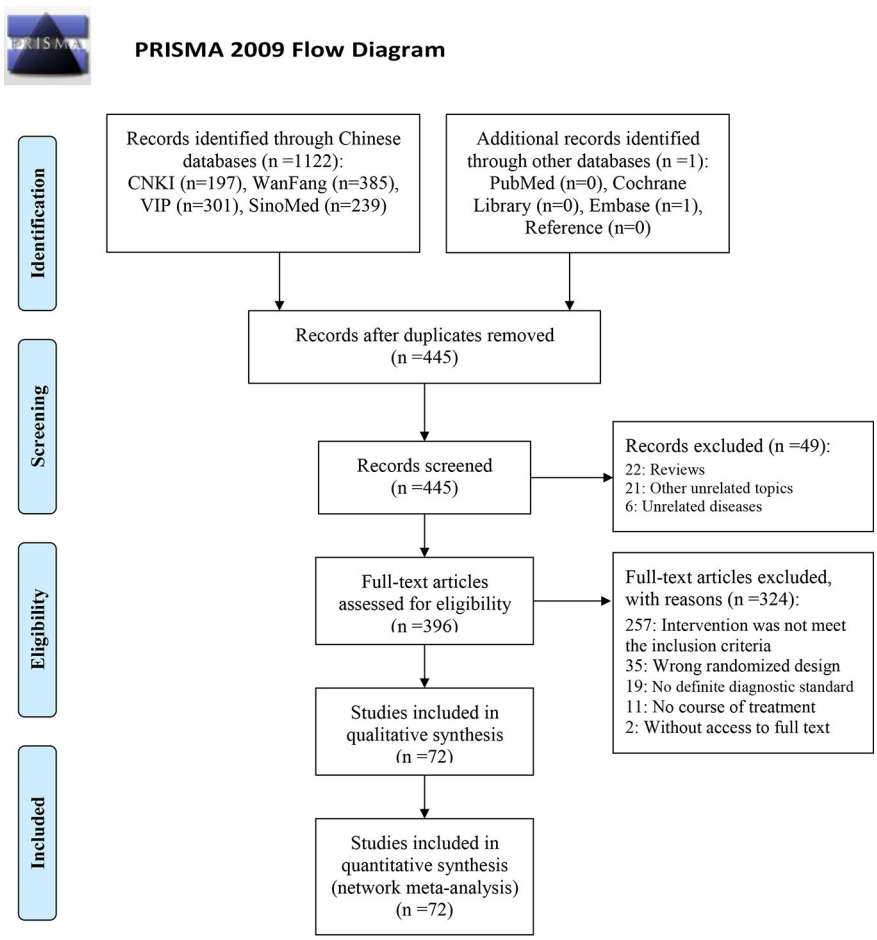

FIGURE 1 | PRISMA flow diagram.

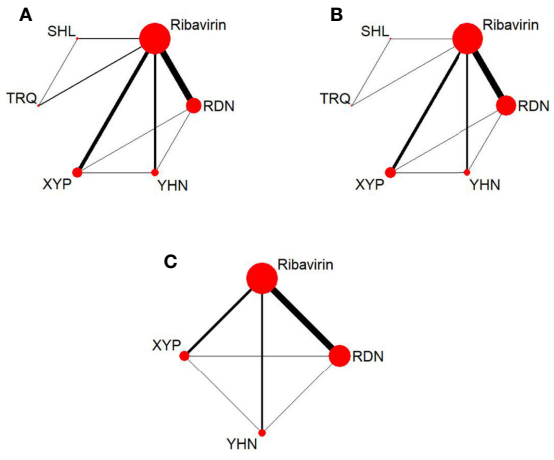

FIGURE 2 | Network graph for different outcomes. **(A)** Total clinical effectiveness; **(B)** antipyretic time; **(C)** Blebs disappearing time. RDN, Reduning injection; SHL, Shuanghuanglian injection; TRQ, Tanreqing injection; XYP, Xiyanping injection; YHN, Yanhuning injetion.

baseline characteristics of the two groups were comparable at the time of grouping, which may have impacted the results, and other corresponding biases were evaluated as "high risk." The risk of bias entries for the remaining studies was rated as "unclear" due to insufficient information. The results of the risk of bias evaluation are shown in **Figure 3**.

Network Meta-Analysis

Total Clinical Effectiveness

Sixty-nine RCTs reported the total clinical effectiveness, involving five CHIs and six interventions. The network graph is shown in **Figure 2**. The OR value of the NMA is shown in **Table 2**. Compared with ribavirin treatment, RDN, SHL, TRQ, XYP, and YHN were found to have greater total clinical effectiveness in patients with herpangina; the between-group differences were statistically significant. There were no significant differences between the remaining intervention groups.

TABLE 2 | Statistical results of network meta-analysis for the outcomes [odds ratio (OR)/mean difference (MD) value, 95% CI].

	Clinical total efficiency*	Antipyretic time	Blebs disappearance time
RDN vs.			
SHL	1.02 (0.51,2.08)	−0.27 (−3.73,2.70)	–
TRQ	1.00 (0.54,1.84)	−0.34 (−3.56,2.76)	–
XYP	0.75 (0.51,1.13)	−0.26 (−1.06,0.58)	−0.09 (−1.03,0.81)
YHN	0.80 (0.50,1.28)	−0.50 (−1.39,0.41)	−0.42 (−1.44,0.64)
Ribavirin	**0.18 (0.14,0.23)**	**−1.33 (−1.82,−0.80)**	**−1.49 (−1.92,−1.06)**
SHL vs.			
TRQ	0.98 (0.47,2.04)	−0.02 (−3.77,3.51)	–
XYP	0.73 (0.36,1.53)	0.05 (−3.00,3.41)	–
YHN	0.79 (0.36,1.69)	−0.17 (−3.28,3.17)	–
Ribavirin	**0.18 (0.09,0.34)**	−1.00 (−3.98,2.33)	–
TRQ vs.			
XYP	0.75 (0.39,1.42)	0.08 (−3.04,3.35)	–
YHN	0.80 (0.39,1.59)	−0.14 (−3.34,3.08)	–
Ribavirin	**0.18 (0.10,0.31)**	−0.98 (−4.04,2.21)	–
XYP vs.			
YHN	1.07 (0.63,1.79)	−0.25 (−1.21,0.76)	−0.33 (−1.62,0.99)
Ribavirin	**0.24 (0.17,0.33)**	**−1.07 (−1.73,−0.42)**	**−1.40 (−2.24,−0.56)**
YHN vs.			
Ribavirin	**0.23 (0.15,0.35)**	**−0.82 (−1.61,−0.08)**	**−1.08 (−2.04,−0.12)**

**indicates that the result is OR; Bold results indicate statistically significant differences between groups; RDN, Reduning injection; SHL, Shuanghuanglian injection; TRQ, Tanreqing injection; XYP, Xiyanping injection; YHN, Yanhuning injection*

The SUCRA ordering and probability value results (**Figure 4**, **Table 3**) indicate that RDN is the most likely to improve total clinical effectiveness in herpangina patients compared with ribavirin, followed by SHL and TRQ.

Antipyretic Time

Forty-five RCTs reported antipyretic time, involving five kinds of CHIs and six interventions. The network diagram is shown in **Figure 2**. The results of NMA (**Table 2**) showed that RDN, XYP, and YHN can shorten the antipyretic time compared with ribavirin; between-group differences in this respect were statistically significant. The difference between the remaining interventions was not statistically significant. The SUCRA ordering and probability value results (**Figure 4**,

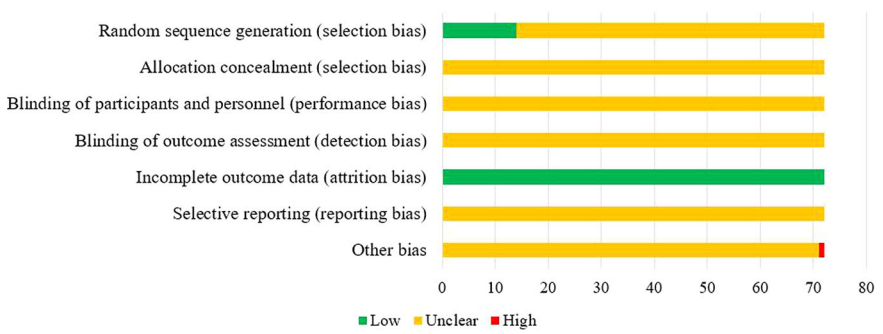

FIGURE 3 | Assessment of risk of bias.

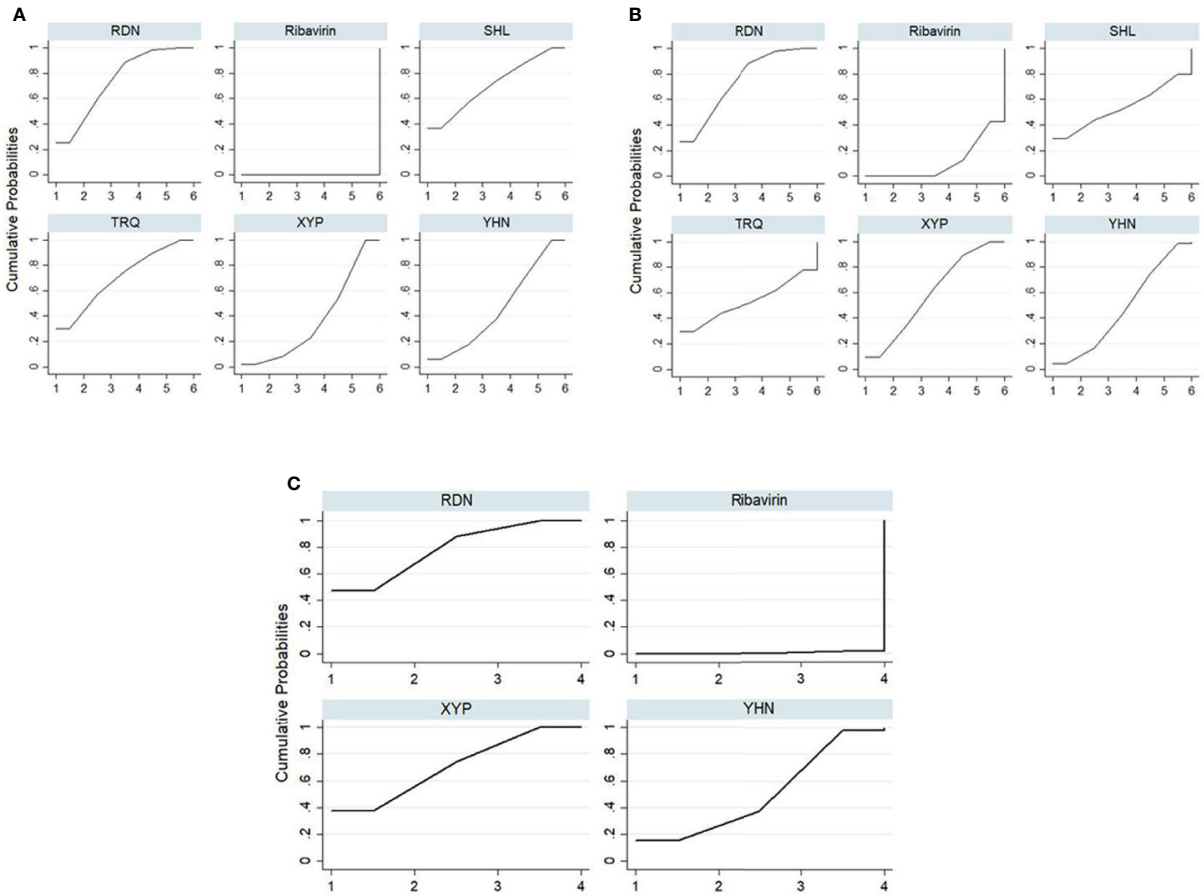

FIGURE 4 | Plot of the surface under the cumulative ranking curves for all treatments. **(A)** Total clinical effectiveness; **(B)** antipyretic time; **(C)** Blebs disappearing time. RDN, Reduning injection; SHL, Shuanghuanglian injection; TRQ, Tanreqing injection; XYP, Xiyanping injection; YHN, Yanhuning injection.

Table 3) indicated that RDN has the best treatment effect, followed by XYP and SHL.

Blebs Disappearing Time

Thirty-eight RCTs reported the blebs disappearing time; these involved four interventions (RDN, XYP, YHN, and ribavirin). The network diagram is shown in **Figure 2**. On NMA (**Table 2**), RDN, XYP, and YHN were found to be associated with a shorter blebs disappearing time compared with ribavirin; the between-group difference in this respect was statistically significant. No significant between-group differences were observed for other interventions. The SUCRA ordering and probability value results (**Figure 4**, **Table 3**) indicated that RDN has the best treatment effect, followed by XYP and YHN.

Cluster Analysis

The cluster analysis method allowed for a comprehensive comparison of the effects of different interventions on total clinical effectiveness, antipyretic time, and blebs disappearing time. The results showed (**Figure 5**) that RDN was the best intervention in terms of total clinical effectiveness and antipyretic time, total clinical effectiveness and blebs disappearing time; these findings suggest that the efficacy of RDN in the treatment of herpangina is worthy of attention.

TABLE 3 | Surface under the cumulative ranking probabilities (SUCRA) results of three outcomes.

Interventions	RDN	SHL	TRQ	XYP	YHN	Ribavirin
Total clinical effectiveness	74.5%	71.3%	70.6%	37.4%	46.2%	0%
Antipyretic time	74.9%	53.9%	53.1%	59.6%	47.5%	11.1%
Blebs disappearance time	78.6%	–	–	70.5%	50.5%	0.5%

The warmer the color, the greater the SUCRA, and the greater the probability of becoming the best intervention.

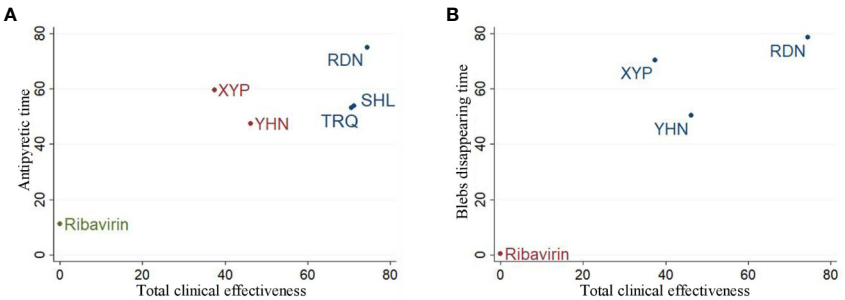

FIGURE 5 | Cluster analysis plot for three outcomes. **(A)** Cluster analysis plot of total clinical effectiveness and antipyretic time; **(B)** cluster analysis plot of Total clinical effectiveness and blebs disappearing time. Interventions with the same color belonged to the same cluster, and interventions located in the upper right corner indicate optimal therapy for two different outcomes; RDN, Reduning injection; SHL, Shuanghuanglian injection; TRQ, Tanreqing injection; XYP, Xiyanping injection; YHN, Yanhuning injection.

Publication Bias

Figure 6 shows the comparison-correction funnel plot for total clinical effectiveness to assess potential publication bias. The points on both sides of the centerline of the funnel plot are not completely symmetrical, and there is a large angle between the correction guideline and the centerline. This suggests that our results may have been affected by publication bias to some extent.

Consistency Test

To evaluate the consistency of each closed loop, the IF and its 95% CI were calculated using Stata software. When the 95% CI contained 0, it was considered to be consistent; otherwise, there was a significant inconsistency in the closed loop. For example, an inconsistency plot of total clinical effectiveness is shown in **Figure 7**. The inconsistency test results showed the inclusion of five rings, and only the 95% CI of 1 ring did not contain 0; this indicates that there was a small inconsistency in the included studies and that the results were relatively reliable.

Adverse Drug Reactions/Adverse Drug Events

Of the 72 included studies, 18 (25.00%) did not monitor ADRs/ADEs during treatment. Out of the 54 (75.00%) studies that described ADRs/ADEs, 22 studies recorded no ADRs/ADEs, while 32 studies reported the occurrence and the number of affected patients in detail. The total number of patients who experienced ADRs/ADEs was 6,647, which accounted for 77.36% of the total patients. No ADRs/ADEs on TRQ were

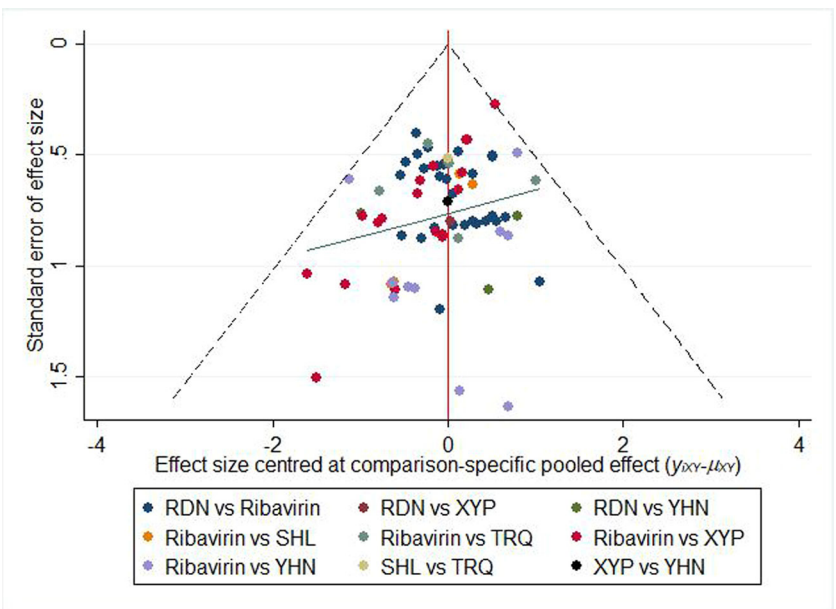

FIGURE 6 | Funnel plot of the clinical effectiveness. RDN, Reduning injection; SHL, Shuanghuanglian injection; TRQ, Tanreqing injection; XYP, Xiyanping injection; YHN, Yanhuning injection.

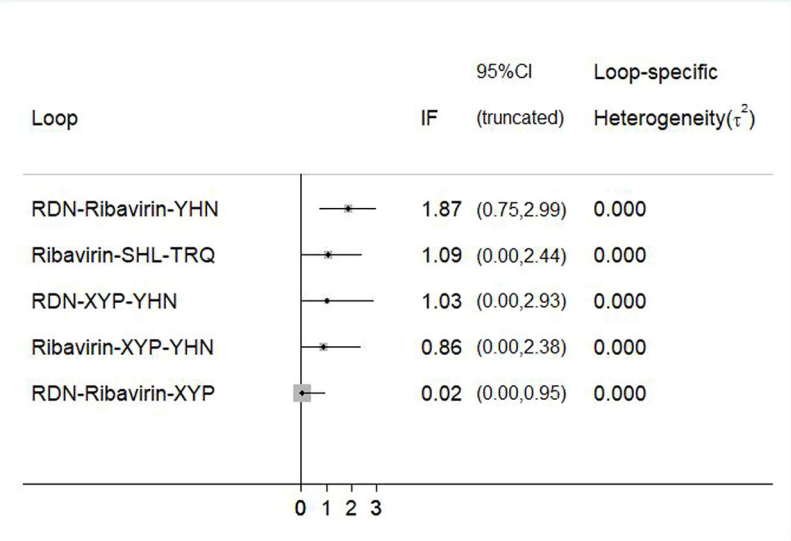

FIGURE 7 | Inconsistency test for the clinical effectiveness. RDN, Reduning injection; SHL, Shuanghuanglian injection; TRQ, Tanreqing injection; XYP, Xiyanping injection; YHN, Yanhuning injection.

reported in the currently included studies; ADRs/ADEs of other interventions are shown in **Table 4**.

DISCUSSION

In this study, we evaluated the use of five types of commonly used CHIs (RDN, SHL, TRQ, XYP, YHN) and ribavirin for the treatment of herpangina. The efficacy of the CHIs was systematically evaluated based on the results of 72 included studies and three outcomes. The results of NMA indicated that the efficacy of RDN, XYP, and YHN was better than that of ribavirin with respect to all outcome measures. With respect to total clinical effectiveness, the efficacy of SHL and TRQ was better than that of ribavirin, and the between-group difference was statistically significant. From the results of SUCRA ordering, among the three outcome indicators, RDN ranked as the best intervention, while all CHIs showed better efficacy than ribavirin. On cluster analysis, RDN was found to be the best intervention with respect to all three outcome measures. Our results highlight the efficacy of RDN in the treatment of herpangina. However, the effect of publication bias on our results cannot be ruled out; therefore, treatment decision-making in individual cases should be guided by specific situations and the experience of clinicians.

TABLE 4 | Details of adverse drug reactions (ADRs)/adverse drug events (ADEs).

	Reduning injection	Shuanghuanglian injection	Xiyanping injection	Yanhuning injection	Ribavirin	Total number of cases
Gastrointestinal reaction	1.65% (27/1,641)	1.33% (2/150)	0.72% (8/1,110)	1.71% (10/586)	0.33% (10/3,056)	57
Rash	0.24% (4/1,641)	1.33% (2/150)	0.81% (9/1,110)	1.71% (10/586)	0.65% (20/3,056)	45
Facial flushing			0.18% (2/1,110)	0.34% (2/586)		4
Gastrointestinal reaction with Rash			0.18% (2/1,110)		0.23% (7/3,056)	9
Leukopenia	0.06% (1/1,641)				1.24% (38/3,056)	39
Increased white blood cell count					0.46% (14/3,056)	14
Anemia					0.07% (2/3,056)	2
Breathing suffering, mild chest pain					0.03% (1/3,056)	1
Total	1.95% (32/1,641)	2.67% (4/150)	1.89% (21/1,110)	3.75% (22/586)	3.01% (92/3,056)	171

In terms of safety, 75% of the included studies monitored ADRs/ADEs. Compared with the medication monitoring of other common respiratory diseases, the RCTs included in this study were better with regard to monitoring the safety of drug use. Among the patients monitored, no significant ADRs occurred in patients treated with TRQ; therefore, its safety needs to be further confirmed by observational studies. In the reported ADRs/ADEs, except for one case of dyspnea and mild chest pain in the ribavirin group, no serious cases occurred in the other groups. The most frequently reported ADRs/ADEs of CHIs were gastrointestinal reactions, followed by rash and leukopenia. Leukopenia occurred primarily in the ribavirin group. The incidence of ADRs was most common in the YHN group, followed by the ribavirin group; the XYP group had the lowest incidence of ADRs/ADEs. Therefore, due care should be taken to avoid ADRs, especially when using YHN and ribavirin.

This is the first study that used the NMA method to evaluate the efficacy and safety of CHIs in the treatment of herpangina and ranked the results of clinical total effectiveness and the disappearing time of two main clinical symptoms. The objective was to provide evidence and recommendations for the clinical selection of drugs. However, some limitations of this study should be considered when interpreting our results: (1) The methodological quality of the included studies was not very high. Only 14 of the 72 RCTs described the correct generation of random sequences. None of the studies mentioned allocation concealment and blinding, and one study did not describe whether the two groups had comparable baseline characteristics. (2) All the included studies were published in Chinese journals; therefore, the findings may not be entirely generalizable to other settings. (3) Most of the included RCTs compared CHIs versus ribavirin, and there was a lack of a more direct comparison of two or more CHIs. (4) This meta-analysis has not been registered online.

Based on the above limitations, we make the following recommendations: (1) For future clinical RCTs, the registration of the protocol should be carried out in advance, and the study should strictly adhere to the protocol to ensure transparency of the implementation process and avoid selective reporting. (2) Future studies should use robust methods for random sequence generation (such as the use of a random number table), implement allocation concealment (e.g., with the use of opaque envelopes), and implement strict blinding to ensure the reliability of the results. (3) More studies should be conducted to evaluate the efficacy of CHIs.

CONCLUSION

In conclusion, the use of CHIs was associated with improved treatment performance and could be beneficial for patients with herpangina compared to ribavirin. RDN showed the best efficacy with respect to all three outcome measures. However, more direct comparison studies of two or more CHIs are needed to further confirm the results. Future studies should include meticulous monitoring of the safety of CHIs.

AUTHOR CONTRIBUTIONS

JW and XD done conception and design of the network meta-analysis. XD, HW and KW performed the network meta-analysis. XD, WZ and XL assessed the quality of the network meta-analysis. XD, HW and KW analyzed study data. XD and HW wrote the paper. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

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PRESENTATION FILE | This file contains three parts, which includes items regarding the PRISMA checklist for network meta-analysis and corresponding pages of this study, the search strategy of traditional Chinese medicine injections in PubMed database, and details about the product information of five CHIs.

REFERENCES

Cai, C. Y. (2011). Clinical Observation of Reduning Injection in Treating Herpangina in Children. *Strait Pharm. J.* 23 (6), 187–188.

Cai, H. B. (2012). Treating 108 Cases of Herpangina with Tanreqing Injection. *Chin. J. Pract. Med.* 39 (12), 114.

Cao, X. C. (2008). Clinical Effect of Shuanghuanglian Injection in Child Herpangina. *China Foreign Med. Treat.* 2791 (15), 99.

Cao, Y. (2015). Clinical Observation of Xiyanping Injection in the Treatment of Herpangina. *J. Yichun Coll.* 37 (12), 82–83.

Chen, G. B., Lin, S. Z., and Ye, Z. L. (2008). Evaluation of the efficiency and safety of Xiyanping in treating Herpangina of children. *Int. Med. Health Guid. News* 14 (12), 82–85.

Chen, H. J. (2012). Clinical Observation of Reduning in Treating Herpangina in Children. *China Pract. Med.* 7 (29), 178–179.

Deng, Z. L., and Tang, D. S. (2014). Discussion on Reduning Injection in the treatment of herpangina. *Med. Aesthetics Cosmetol.* 3 (5), 554–555.

Dong, C. Y. (2014). Clinical analysis of treatment of herpangina in children with Reduning. *For All Health* 8 (2), 196.

Dong, X. J., and Feng, T. (2013). Therapeutic effect of Yanhuning injection on herpangina. *Guide China Med.* 11 (33), 207–208.

Fang, Y. (2011). Therapeutic effect of Yanhuning on children with herpetic angina. *Mod. J. Integr. Tradit. Chin. West. Med.* 20 (5), 584–610.

Feng, C. X., and Peng, S. H. (2013). Treating 80 Cases of Herpangina in Children with Tanreqing Injection. *Guiding J. Tradit. Chin. Med. Pharm.* 19 (5), 101–102.

Feng, J. H., Zhang, Y. S., and Feng, X. C. (2015). Clinical efficacy of Reduning Combined with Ribavirin in the treatment of herpangina. *J. China Prescript. Drug* 13 (10), 90–91.

Ge, L., Pan, B., Pan, J. X., Li, Y. N., Wu, Y. T., Guo, S. J., et al. (2017). An Introduction of AMSTAR-2 : A Quality Assessment Instrument of Systematic Reviews Including Randomized or Non-randomized Controlled Trials or Both. *Chin. J. Drug Eval.* 34 (5), 334–338.

Guo, Q., and Li, S. S. (2019). Recombinant Human Interferon α-1b Spray in the Treatment of Children with Herpangina. *J. Pedia. Pharm.* 25 (2), 31–33.

Guo, Y. B., Gui, J. G., Wu, H. Z., and Cui, Y. C. (2014). Therapeutic effect of Reduning injection on Yanhuning in the treatment of herpes angina. *Clin. J. Tradit. Chin. Med.* 26 (6), 586–587.

Guo, J. H. (2009). Therapeutic effect of Xiyanping on children with herpangina. *Public Med. Forum Mag.* 13 (S1), 133–134.

Guo, Q. J. (2010). Therapeutic effect of Reduning injection on 60 cases of herpangina. *Mod. J. Integr. Tradit. Chin. West. Med.* 19 (8), 933–934.

Guo, L. J. (2011). Yanhuning for the treatment of herpes angina 44 cases. *Chin. Med. Mod. Dis. Educ. China* 9 (15), 19.

He, M. L., and Peng, F. (2010). Therapeutic effect of Xiyanping injection on herpetic angina. *Chin. J. Misdiagn.* 10 (10), 2349–2350.

Higgins, J., and Green, S. E. (2010). Cochrane handbook for systematic reviews of interventions Version 5.1.0. *Cochrane Collab.*, 202–206.

Hu, Z. S. (2008). Evaluation of the efficacy of Yanhuning in the treatment of herpetic angina. *China Contemp. Med.* 14 (13), 138–139.

Hu, W. X. (2014). Application of Reduning Injection in Pediatric Herpangina. *Neimonggu J. Tradit. Chin. Med.* 33 (15), 9–10.

Huang, L. M., Meng, X. P., Meng, S. Y., Li, Z. P., and Hu, F. E. (2008). Analysis of 68 cases of herpes angina treated with Xiyanping. *J. Chin. Mod. Pediatr.* 5 (1), 58–59.

Huang, R. (2016). Clinical observation of riboflavin sodium phosphate combined with interferon in treatment of children herpangina. *Drugs Clin.* 31 (2), 186–189.

Hutton, B., Salanti, G., Caldwell, D. M., Chaimani, A., Schmid, C. H., Cameron, C., et al. (2015). The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions: Checklist and Explanations. *Ann. Intern. Med.* 162 (11), 777–784. doi: 10.7326/M14-2385

Ji, X. Z., Chen, Z. H., and Chen, X. J. (2014). Clinical Observation on 190 Cases of Reduning Injection in Children with Herpangina. *Med. Info.* 15 (39), 401.

Jia, M., and Tian, L. Y. (2012). Therapeutic effect of Xiyanping injection on 70 cases of herpetic angina. *Xinli Yesheng* 1 (8), 281.

Jiang, Z. F., Shen, K. L., Shen, Y., and Zhu, F. T. (2015). *Practical pediatrics. Eighth edition* (Beijing: People's Medical Publishing House), 901–902.

Jiang, Z. E. (2009). Clinical Observation on Treatment of Herpangina with Tanreqing Injection. *J. Liaoning Coll. Tradit. Chin. Med.* 11 (6), 148.

Ke, H. Y. (2014). Clinical research of Reduning Injection in the treatment of children with herpetic angina. *J. Pediatr. Tradit. Chin. Med.* 10 (5), 18–20.

Li, Q. X., and Zhan, H. Y. (2017). Clinical Study of the Effects and Mechanism of Qingfei Liyan Decoction Combined with Acupoint Exter-nal Application on Herpangina in Children. *J. Emerg. Tradit. Chin. Med.* 26 (6), 1022–1025.

Li, J. Y., Dai, Z. L., and Gao, C. H. (2011). Application of Xiyanping injection on Herpesangina. *China Pract. Med.* 6 (14), 30–31.

Li, X. P. (2011). Therapeutic effect of Yanhuning on herpes angina. *World Health Digest* 8 (7), 380–381.

Li, H. B. (2012). Clinical observation of Yanhuning in treating 42 cases of herpetic angina in children. *Jilin Med. J.* 33 (1), 132–133.

Lin, J. Y. (2014). Treatment of 48 cases of herpetic angina with Xiyanping injection. *China Naturopathy* 22 (7), 44–45.

Liu, A. L., and Li, L. (2011). Therapeutic effect of Xiyanping injection and Reduning injection on herpes angina. *Chin. J. Clin.* 5 (4), 1152–1154.

Liu, J., Xiong, Z. Y., and Ao, X. D. (2019). Clinical analysis of treatment of herpangina with different administration routes and different doses of interferon. *Contemp. Med.* 25 (5), 146–147.

Liu, W. (2015). Therapeutic effect of Reduning on the treatment of herpangina in children. *Yiyao Qianyan* 5 (30), 109–110.

Lv, J. (2009). Clinical Observation on 30 Cases of Herpangina Treated by Yanhuning. *J. Commun. Doctors* 11 (13), 112.

Pang, G. X., Lin, S. Z., and Zhang, Z. H. (2008). Evaluation of the efficiency and safety of Redunin injection in treating Herpangina of children. *Neimonggu J. Tradit. Chin. Med.* 20 (10), 8–9.

Peng, Y. L., and Tao, Y. (2010). Clinical Analysis of Shuanghuanglian Freeze-dried Powder Injection in Treating 66 Cases of Herpangina in Children. *J. North China Coal Med. Coll.* 12 (6), 825.

Pu, H. X. (2012). Therapeutic effect of Reduning injection on children with herpangina. *Yiyao Qianyan* 2 (24), 185–186.

Qu, D. Y., Li, Z. F., and Liu, Z. L. (2016). Therapeutic effect of Yanhuning on herpes angina. *World Latest Med. Info.* 16 (73), 139.

Shen, X. Y. (2010). Clinical Observation on 50 Cases of Herpangina in Treating Xiyanping. *China Health Care Nutr.* 2 (10), 139–140.

Song, X. G., and Fan, J. (2013). Therapeutic effect of Yanhuning injection on herpes simplex angina in infants. *Guide China Med.* 11 (11), 273–274.

Su, Z. Q., and Ke, D. H. (2012). Changes of immune function in children with herpangina and observation of curative effect of Xiyanping. *Strait Pharm. J.* 24 (9), 132–133.

Sun, L. M., Chang, X. C., Zhao, R. B., Cui, Y. L., and Wang, L. J. (2011). Reduning treatment of children with herpes angina efficacy. *China Med. Herald* 8 (6), 149–150.

Tan, G. M. (2011). Therapeutic Efficacy of Tanreqing Injection for Herpangina. *Guangming J. Chin. Med.* 26 (6), 1137–1138.

Tan, F. Z. (2014). Therapeutic effect of Reduning on the treatment of herpangina in children. *Cardiovasc. Dis. J. Integr. Tradit. Chin. West. Med.* 2 (7), 87–88.

Wang, Z. Y., and Li, J. (2015). Therapeutic effect of Reduning on herpangina. *World Latest Med. Info.* 4 (41), 134–136.

Wang, Z. J., Xiang, J. J., and Pang, W. J. (2012). Clinical application of Yanhuning in herpangina. *J. China Tradit. Chin. Med. Info.* 4 (1), 64.

Wang, H. R. (2012). Clinical Observation on 92 Cases of Herpangina in Children Treated with Reduning Injection. *Chin. Commun. Doct.* 14 (32), 174–176.

Wang, H. Y. (2012). Clinical Observation on Treatment of Herpangina in Children with Traditional Chinese Medicine Injection. *Guide China Med.* 10 (5), 232–233.

Wang, L. X. (2013). Therapeutic effect of Shuanghuanglian injection on children with herpetic angina. *Med. Info.* 26 (2), 160–161.

Wang, Y. M. (2013). Application of Xiyanping in the treatment of herpangina. *World Health Digest* 6 (40), 83–84.

Wei, K. C. (2007). Therapeutic effect of Yanhuning on herpes angina. *Youjiang Med. J.* 35 (6), 668–669.

Wen, J. F. (2012). Therapeutic effect of Tanreqing injection on herpetic angina. *Health Guide* 2 (2), 1–2.

Wu, Q. (2018). Efficacy and safety of treatment of Herpangina with elevated C-reactive protein in children. *J. Clin. Med. Lit.* 5 (97), 31–32.

Xia, J. W. (2016). Observation on the Therapeutic Effect of Xiyanping Injection in Treatment of Herpes. *China Continuing Med. Edu.* 8 (30), 173–175.

Xia, W. H. (2016). Meta analysis of curative effect of reduning injection in the treatment of herpangina in children. *Chin. Commun. Doct.* 138-139 (34), 113.

Xiao, X. L. (2010). Effective observation on treating pediatric herpetic angina with Redu-ning. *Clin. J. Chin. Med.* 2 (13), 50.

Xiao, X. Q. (2016). Influence of Reduning injection on immune function in children with blister measles angina. *Int. Med. Health Guid. News* 22 (20), 3141–3143.

Xie, B. Q. (2011). Therapeutic effect of Reduning injection on children with herpangina. *J. China Tradit. Chin. Med. Info.* 3 (7), 124.

Xie, J. (2017). Clinical Observation of Reduning in Treating Herpangina in Children. *Oriental Diet Ther. Health Care* 10 (4), 132.

Xu, X. Y., Hu, Y., Xue, J., Xu, W. Q., Xu, X. L., and Dai, T. J. (2009). Efficacy and safety of Reduning injection in children with herpangina. *World Clin. Drugs* 30 (10), 609–611.

Xu, J. L., Liang, Z. R., Guo, Z. L., Li, Z. C., and Deng, L. M. (2016). Xiyanping Combined with Ribavirin in Treatment of Children with Pediatric Herpangina. *Chin. J. Exp. Tradit. Med. Form.* 22 (4), 195–200.

Yang, M. L., Tang, H., and He, H. (2013). Observe the efficacy of Xiyanping injection in treatment of children with herpangina in 123 cases. *Natl. Med. Front. China* 8 (22), 63–64.

Yang, L. (2011). Clinical observation of Xiyanping in the treatment of 61 cases of herpetic angina in children. *World Health Digest* 8 (36), 182.

Yang, L. N. (2013). Therapeutic effect of Reduning on the treatment of herpangina in children. *China Hwalth Care Nutr.* 2 (11), 475.

Yang, F. J. (2014). Effect of Yanhuning on the treatment of herpangina in children. *China Pract. Med.* 9 (35), 99–100.
Yin, Z. X. (2011). Therapeutic effect of Yanhuning on herpes angina. *J. China Tradit. Chin. Med. Info.* 3 (21), 129.
Yu, J. T., and Qian, C. Y. (2014). Clinical Observation of Reduning in Treating Herpangina in Children. *Acta Chin. Med. Pharm.* 42 (2), 127–128.
Zeng, H. J., Li, J., and Huang, Y. B. (2013). To explore the efficacy of Xiyanping injection in the treatment of herpes angina. *Healthmust-Readmagazine* 12 (1), 293–294.
Zeng, Y. (2011). Therapeutic effect of Reduning and ribavirin on children with herpangina. *Med. Info.* 24 (12), 780.
Zhang, S. C., Liu, T., and Zhang, W. P. (2012). Clinical observation on Reduning Injection in the treatment of 96 children with herpes angina. *China Modern Med.* 19 (27), 25–26.
Zhang, F. R. (2011). Clinical analysis of Xiyanping injection in the treatment of herpetic angina. *Mod. Med. Health* 27 (18), 2832.
Zhang, L. (2011). Therapeutic effect of Xiyanping on children with herpetic angina. *Gansu. Med. J.* 30 (3), 164–165.
Zhang, Z. M. (2012). Clinical Observation of Reduning Injection in Treating Herpangina in Children. *J. Med. Theory Pract.* 25 (12), 1481–1482.
Zhang, Y. L. (2013). Therapeutic effect of Reduning injection on children with herpangina. *Yiyao Qianyan* 3 (6), 164–165.
Zhao, B. Y. (2012). Clinical treatment of herpetic angina in children. *Guide China Med.* 10 (31), 547–548.
Zhou, S. Y. (2012). Therapeutic effect of Reduning injection combined with Yanhuning injection on herpes angina. *China Mod. Doctor* 50 (11), 142–144.
Zhou, H. (2013). Clinical Observation of Xiyanping Injection in Treating Pediatric Herpangina. *Med. Info.* 26 (8), 418–419.
Zhu, Q. L., Yang, S. P., Ye, X. H., and Chen, H. (2014). A Systematic Review of Yanhuning versus Ribabirin for Herpangina in Children. *J. Pediatr. Pharm.* 20 (4), 4–9.
Zhu, J. M. (2013). Therapeutic effect of Tanreqing injection on children with herpetic angina. *Med. Info.* 14 (21), 316–317.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Efficacy and Safety of Direct-Acting Antiviral Therapy in Patients With Chronic Hepatitis C Virus Infection: A Real-World Single-Center Experience in Tianjin, China

Huan Xia[1†], *Chengzhen Lu*[2†], *Yin Wang*[3], *Silvere D. Zaongo*[1,4], *Yue Hu*[1], *Yue Wu*[1], *Zhongfang Yan*[5] *and Ping Ma*[1*]

[1] Department of Infectious Disease, Tianjin Second People's Hospital, Tianjin, China, [2] Department of Hepatology, Tianjin Second People's Hospital, Tianjin, China, [3] Department of Infectious Disease, Public Health Clinical Center of Chengdu, Chengdu, China, [4] International School of Medicine, Tianjin Medical University, Tianjin, China, [5] Tianjin Second People's Hospital, Tianjin, China

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Yonggang Zhang,
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Italian Society of Pharmaceutical Medicine, Italy
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University of Tripoli, Libya

***Correspondence:**
Ping Ma
mapingtianjin@163.com

[†]These authors have contributed equally to this work

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Objective: Toward the limited real-world data concerning the treatment response to brand direct-acting antiviral agents (DAAs) therapy, we proposed to evaluate the efficacy and safety of DAAs for the treatment of chronic hepatitis C virus (HCV) in mainland China.

Methods: In this retrospective, single-center, cohort study, all HCV-infected adult patients treated with brand DAA drugs covered by Tianjin local health insurance (Apr 2018–Sept 2019) and responding to other specific inclusion criteria were recruited. The five available DAA regimens included sofosbuvir + ribavirin (SOF + RBV), elbasvir/grazoprevir (EBR/GZR), ombitasvir/paritaprevir/ritonavir/dasabuvir (OBV/PTV/r/DSV) ± RBV, daclatasvir + asunaprevir (DCV + ASV), and SOF + DCV ± RBV. Demographic, virologic, clinical, and adverse effects data obtained during and after DAAs treatment were collected. We evaluated the rate of sustained virological response at 12 weeks post-treatment (SVR12), the incidence of adverse effects, and assessed the factors associated with SVR12.

Results: Four hundred ninety-four patients finished the treatment and completed the 12-week post-treatment follow-up. The overall SVR12 rate was estimated at 96.96%. SVR rates greater than 95% were achieved in most of the HCV genotypes with the exception of GT1a (0%), GT3a (93.33%), and GT3b (88.24%). SVR12 for patients treated with DCV + ASV, EBR/GZR, OBV/PTV/r/DSV ± RBV, SOF + DCV ± RBV, and SOF + RBV for 12 or 24 weeks was 86.67%, 100%, 98.11%, 97.56%, and 95.06%, respectively. Subjects with compensated cirrhosis (92.73%) and prior treatment experience (77.78%) had significantly lower SVR rates when compared to chronic hepatitis C (98.15%) and treatment-naive (97.69%) groups. In Tianjin, the available DAA regimens were generally well-tolerated, and not a single serious adverse event was reported.

Conclusion: In this large real-life single-center HCV cohort from China, oral DAAs were highly effective and well-tolerated. Further and larger-scale studies are needed to evaluate their clinical safety and efficacy.

Keywords: direct acting antivirals, hepatitis C, real-world experience, DAAs, HCV, China

INTRODUCTION

Globally, chronic hepatitis C virus (HCV) infection is a significant challenge to public health. In 2015, an estimated 71 million people were infected with HCV worldwide, and there were approximately 9.8 million HCV viremic people in China (Polaris Observatory, 2017). There, the infection is showing a significant increase in some provinces (Liu et al., 2018). In fact, according to the latest report from Tianjin Centers for Disease Control and Prevention, the incidence of chronic HCV in Tianjin was 5.87/100,000 in 2018, which was as much as 1.4-fold higher compared to the numbers estimated in 2016. In China, HCV is much more prevalent among older people (Liu et al., 2018), who are more likely to experience chronic liver disease. Long-term HCV infection is a leading cause of hepatic inflammation, extensive fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and liver-related death (Polaris Observatory, 2017). The HCV-related diseases represent an immense health and economic burden in China.

The introduction of direct-acting antiviral agents (DAAs), with their high rates of sustained virological response (SVR) (European Association for the Study of the Liver, 2018), has revolutionized the management of chronic HCV infection. Thanks to DAAs, HCV can now be cured in most patients, even in those with advanced cirrhosis (Feld et al., 2015; Forns et al., 2017), genotype (GT) 3 (Kwo et al., 2017), and history of prior treatment failures (Feld et al., 2015; Lawitz et al., 2017). But the application of brand DAA drugs is limited in most regions of mainland China due to their expensive cost, different treatment guidelines, and reimbursement policies established by local governments (Bian et al., 2017). As a result, generic HCV drugs hold a high leading position in China. Fortunately, since April 2018, Tianjin local health insurance can cover HCV treatment (Bureau, 2018). Furthermore the brand DAAs used for the treatment include sofosbuvir (SOF), elbasvir/grazoprevir (EBR/GZR), ombitasvir, paritaprevir, ritonavir, dasabuvir (OBV/PTV/r/DSV), daclatasvir (DCV), and asunaprevir (ASV). Soon, the recently licensed SOF/velpatasvir (SOF/VEL) will be added to the reimbursement drug list. Tianjin health insurance reimburses up to $5,660 per HCV patient, which accounts for 85–90% of the cost (Bureau, 2018). The favorable reimbursement policy and early access to DAAs in Tianjin constitute a perfect condition to firstly report real-world experience with available brand DAAs in the treatment of Chinese HCV-infected patients.

Thus far, the results of real-world investigations on DAAs efficacy are mostly reported in western countries. In general, they present similar efficacy as observed in clinical trials (Saxena et al., 2017; Berg et al., 2019; Mera et al., 2019). These results also revealed the effectiveness of DAAs in some specific categories of patients (Saxena et al., 2017; Mera et al., 2019). Few real-life data have been reported in Asian countries except in Japan and Korea. Therefore, the purpose of this study was to assess the efficacy and safety of available brand DAAs in a sizeable real-life HCV patients cohort in Tianjin, China.

MATERIAL AND METHODS

Study Population and Antiviral Regimens

In this single-center—Tianjin Second People's Hospital—retrospective real-world cohort study, patients meeting the following inclusion criteria were enrolled: (1) ≥18 years old; (2) a history of chronic HCV infection; (3) HCV GT 1, 2, 3, 6, unknown, or mixed; (4) with or without cirrhosis (compensated and decompensated); (5) treatment-naïve or treatment-experienced with interferon-based regimens; (6) negative results for antinuclear, anti-mitochondria, anti-smooth muscle autoantibodies; (7) with Tianjin local Medical Insurance; (8) treated with available brand DAAs covered by Tianjin local health insurance. Exclusion criteria were as listed: incomplete data, discontinued treatment, or loss during the 12-week post treatment follow-up. Ethical approval was obtained from the human medical ethics committee of Tianjin Second People's Hospital and carried out following the principles of the Helsinki Declaration. Written informed consent was provided by each recruited patient.

At the treatment initiation, DAA containing regimens were chosen based on the current Asian-Pacific Association for the Study of the Liver (APASL) guidelines (Omata et al., 2016). During the study period, the available DAAs approved by Chinese government and covered by Tianjin local medical insurance were: (1) SOF (400 mg once daily) + ribavirin (RBV) daily (1,000 mg or 1,200 mg daily divided into three doses in patients who weighed < 75 kg or > 75 kg, respectively) for 12 or 24 weeks, (2) SOF (400 mg) + DCV (60 mg) ± RBV daily for 12 or 24 weeks, (3) EBR (50 mg)/GZR (100 mg) daily for 12 weeks, (4) OBV/PTV/r (25 mg/150 mg/100 mg once daily) + DSV (500 mg daily, divided into two doses) ± RBV for 12 weeks, and (5) DCV (60 mg) + ASV (100 mg) twice daily for 24 weeks. The use of RBV was determined by physicians depending on the practice guidelines and clinical indications for real-world settings. Patients were treated with different DAA regimens according to their clinical conditions.

Data Collection

Demographic and baseline clinical characteristics, history of previous HCV treatment, and laboratory values were collected from the electronic medical records. The following clinical tests

were completed at initial visits: anti-HCV, HCV RNA, HCV GT, serum hepatitis B virus (HBV) surface antigen, anti-human immunodeficiency virus (HIV), auto-antibodies, liver function, renal function, prothrombin time activity percentage, alpha-fetoprotein (AFP), thyroid function, chest-X ray, electrocardiography, abdominal imaging examinations, and tests of liver stiffness. Laboratory values were collected at baseline, week 12 and 24 [end of treatment (EOT)], then at week 12 after EOT.

Serum HCV RNA was tested using a Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test (Roche Molecular Systems, Branchburg, NJ, USA; version 2.0). Anti-HCV reactivity was examined with a enzyme linked immunosorbent assay (Kehua Biotech, Shanghai, China). The HCV GT was sequenced and identified by a gene-sequencing assay.

Cirrhosis was defined by liver biopsy, whenever available, or based on clinical, laboratory, endoscopic, and radiological findings (i.e., abdominal ultrasound, computed tomography, magnetic resonance imaging, Fibroscan®). Decompensated cirrhosis was defined as the presence or history of variceal bleed, ascites, or hepatic encephalopathy. HCC was screened by at least two imaging tools, or by one imaging diagnostic modality plus a serum AFP level of at least 400 ng/ml.

Evaluations of Efficacy and Safety

The primary efficacy endpoint was sustained virologic response (SVR12, which was defined as an undetectable HCV RNA viral loads < 15 IU/ml at week 12 after EOT). Adverse events (AEs) and serious adverse events that occurred both during and after treatment were recorded by physicians or nurses in charge. AEs related to DAAs therapy was defined as any unintended and unfavorable sign (including abnormal lab finding), symptom, or disease temporally associated with the use of DAA drugs. DAA drugs and these adverse reactions followed a chronological order (after initiation of DAAs treatment). All AEs were classified according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 developed by the US National Cancer Institute (US NCI) (Health, N.I.o, 2017). Based on the tool, the severity of adverse event was classified according to unique clinical descriptions for each event.

Statistical Analysis

Categorical variables were reported as frequencies (percentages); continuous variables were presented as median (interquartile range) or mean (standard deviation) as appropriate. Differences in categorical variables were assessed using the chi-square test or Fisher's exact test. Continuous variables were compared using t-tests or Mann-Whitney *U* test. In the univariate analysis, chi-square and Fisher's test were used for categorical variables when appropriate, and the odds ratio with 95% confidence intervals were calculated for SVR assessment. A two-tailed *P* value < 0.05 was considered statistically significant. All analyses were performed using SAS version 9.4 software (SAS Institute, Cary, NC, USA).

RESULTS

Between April 2018 and September 2019, 694 registered HCV infected Medicare patients were screened. After excluding those without treatment (n = 32), incomplete data (n = 42), discontinued treatment (n = 1), lost to follow up (n = 5), and patients with no post-treatment 12-week follow-up (n = 120): we recruited 494 patients who completed both DAAs treatment and 12-week follow-up compliance.

Characteristics of the Study Population

The demographic and clinical characteristics of the patients were stratified according to SVR12 achievement status. The details are presented in **Table 1**. Overall, the mean age was 53.5 (± 13.07) years, 47.98% (237/494) were male, and 3.64% (18/494) were treated previously. Of the treatment-experienced patients, 72.22% (13/18) had previously received interferon-based regimen (**Table 1**).

From HCV GTs analysis, we noted the following distribution: 71.86% (355/494) of GT1(with 1 of 1a and 354 of 1b), 18.42% (91/494) of GT2 (all were of 2a), 6.46% (32/494) of GT3 (with 15 of 3a and 17 of 3b), 1.42% (7/494) of GT6 (with 4 of 6a, 2 of 6e, and 1 of 6n), and 1.82% (9/494) of unknown or mixed GT (**Figure 1**). More than 20% of the patients had cirrhosis (23.47%, 116/494). Among them, 5.17% (6/116) had a history of decompensated cirrhosis. Ten patients (10) out of 494 had a previous history of HCC (2.02%) while the double of this estimation represented the patients co-infected with HBV or HIV (4.05%, 20/494).

At baseline, median liver stiffness measurement (LSM) was 9 kPa. Besides, mean HCV-RNA and mean estimated glomerular filtration rate (eGFR) according to the MDRD (Modification of Diet in Renal Disease) formula were 5.99 Log_{10} IU/ml and 97.17 ml/min/1.73 m^2, respectively. Most of the patient characteristics were similar in SVR12 achieved or not groups ($p > 0.05$). A statistically significant difference was noted when comparing the presence of cirrhosis ($p = 0.0264$), and prior-treatment experience status ($p < 0.0001$) between the aforementioned groups (see **Table 1**).

The treatment regimens use based on the GT profiles are shown in **Table 2**. Patients with GT1 used either OBV/PTV/r/DSV ± RBV (59.72%), EBR/GZR (17.75%), SOF + RBV (14.37%), SOF + DCV ± RBV (3.94%), or DCV + ASV (4.23%). On the other hand, patients with GT2-6 were either treated with SOF + RBV or SOD/DCV ± RBV while those harboring unknown or mixed GTs only received SOF + RBV.

Treatment Efficacy

There were 494 HCV infected patients with or without liver cirrhosis who completed the treatment. The overall SVR12 rate was estimated at 96.96% (479/494). Concerning HCV GTs, SVR rates greater than 95% were achieved in all GTs with the exception of GT1a (0%, 0/1), GT3a (93.33%, 14/15), and GT3b (88.24%, 15/17). The results revealed 86.67% (13/15), 100% (64/64), 98.11% (208/212), 97.56% (40/41), and 95.06% (154/162) of SVR12 in patients treated with DCV + ASV,

TABLE 1 | Demographic and clinical features based on SVR12 status.

	Total (n = 494)	No SVR12 (n = 15)	SVR12 (n = 479)	*p* value
Age in year, mean (SD)	53.50 (13.07)	55.47 (15.40)	53.44 (13.00)	0.2947
Male, n (%)	237 (47.98)	8 (53.33)	229 (47.81)	0.6732
Prior treatment experienced				
Overall, n (%)	18 (3.64)	4 (26.67)	14 (2.92)	<0.0001
Prior IFN-based regimen, n (%)	13 (2.63)	2 (13.33)	11 (2.30)	0.0554
Cirrhosis, n (%)				0.0264
Compensated	110 (22.26)	8 (53.33)	102 (21.34)	
Decompensated	6 (1.21)	0 (0.00)	6 (1.26)	
History of HCC, n (%)	10 (2.02)	1 (6.67)	9 (1.88)	0.2679
HIV/HCV or HBV/HCV co-infection, n (%)	20 (4.05)	2 (13.33)	18 (3.76)	0.235
Solid organ transplant recipients, n (%)	10 (2.02)	0 (0.00)	10 (2.09)	>0.9999
GT, n (%)				0.2737
GT1	355 (71.86)	9 (60.00)	346 (72.23)	
GT2	91 (18.42)	3 (20.00)	88 (18.37)	
GT3	32 (6.46)	3 (20.00)	29 (6.05)	
GT6	7 (1.42)	0 (0.00)	7 (1.46)	
unknown or mixed	9 (1.82)	0 (0.00)	9 (1.88)	
HCV RNA (Log_{10} IU/ml), mean (SD)	5.99 (0.96)	6.20 (0.75)	5.99 (0.97)	0.314
Liver stiffness measurement (LSM) (kPa), median (IQR)	9.00 (9.00)	13.5 (18)	6 (8)	0.3118
ALT (U/L), median (IQR)	43 (44)	41.5 (26)	43 (45)	0.9603
Bilirubin (μmol/L), median (IQR)	14 (8)	14.5 (6)	14 (8)	0.3903
Albumin (g/L), mean (SD)	43.40 (6.12)	42.50 (5.54)	43.43 (6.14)	0.7132
AFP (ng/ml), median (IQR)	5 (6)	7 (20)	5 (6)	0.3723
eGFR (ml/min/1.73 m^2), mean (SD)	97.17 (24.64)	104.00 (20.45)	97.00 (24.73)	0.588
Platelets (10^9/L), median (IQR)	159 (101)	138 (109)	159 (100)	0.4332
Hemoglobin (g/L), mean (SD)	136.79 (24.14)	139.4 (24.98)	136.7 (24.14)	0.7661

Data expressed as mean (standard deviation) or median (Q3–Q1) or sample size and proportion (%). SD, standard deviation; IQR, interquartile range; SVR, sustained virologic response; IFN, interferon; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; GT, genotype; HCV, hepatitis C virus; HBV, hepatitis B virus; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; eGFR, estimated glomerular filtration rate.

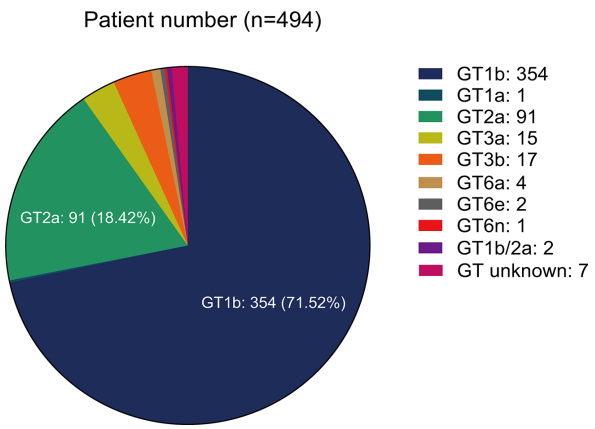

FIGURE 1 | Hepatitis C virus (HCV) genotypes distribution.

EBR/GZR, OBV/PTV/r/DSV ± RBV, SOF + DCV ± RBV, and SOF + RBV for 12 or 24 weeks, respectively (**Figures 2A, B**). Subjects with compensated cirrhosis (92.73%, 102/110) and prior treatment experience (77.78%, 14/18) had relatively lower SVR rates when compared to chronic HCV (98.15%, 371/378) and treatment-naive (97.69%, 465/476) groups (**Figure 2C**). There were 90% and 100% of SVR rate for HBV or HIV co-infected patients (18/20) and transplant recipients (10/10), respectively.

Factors Predicting Failure to Achieve SVR12

Overall, the absence of SVR12 was only associated with LSM (OR 1.043, 95%CI 1.006–1.082, $p = 0.0221$) and AFP (OR 1.002, 95% CI 1.000–1.003, $p = 0.0303$) in univariate analysis (**Table 3**). No differences in age, sex, HCV RNA, history of HCC, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), treatment regimens, and platelets were found between patients achieving or not SVR12. Besides, since GT 1b was the dominant GT, using a logistic regression, univariate and multivariate analyses were conducted to examine their potential influence on SVR12 onset. We concluded that GT1b was not able to predict the failure to achieve SVR12.

Safety and Tolerability

Overall, adverse events were reported in 190 (38.5%) patients (**Table 4**). Fatigue (9.5%), anemia (7.5%), and dizziness (5.9%) were the most commonly encountered and were considered as drug-related in 71 (14.4%) participants. Adverse events ranged mostly between mild and/or moderate. Eleven (11, 2.2%) patients developed anemia due to RBV, which lead us to reduce its dosage. None of the patients developed severe adverse events (leading to discontinuation of the treatment) or died during the treatment (see **Table 4**). No HCC incidence was found throughout the study. Our observations indicated that DAA regimens were safe for HCV-infected patients who tolerated them well.

TABLE 2 | DAA regimens administered to hepatitis C virus (HCV) positive patients according to their genotype.

Regimens	Total	GT1	GT2	GT3	GT6	GT unknown or mixed
SOF + RBV	162 (32.79)	41 (14.37)	76 (83.52)	22 (68.75)	5 (71.43)	9 (100)
SOF + DCV ± RBV	41 (8.30)	14 (3.94)	15 (16.48)	10 (31.25)	2 (28.57)	0 (0.00)
EBR/GZR	64 (12.96)	63 (17.75)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
OBV/PTV/r/DSV ± RBV	212 (42.91)	212 (59.72)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
DCV + ASV	15 (3.04)	15 (4.23)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

Data expressed as sample size and proportion (%).

DAAs, direct-acting antiviral agents; HCV, hepatitis C virus; GT, genotype; SOF, sofosbuvir; RBV, ribavirin; DCV, daclatasvir; EBR/GZR, elbasvir/grazoprevir; OBV/PTV/r/DSV, ombitasvir/paritaprevir/ritonavir and dasabuvir; ASV, asunaprevir.

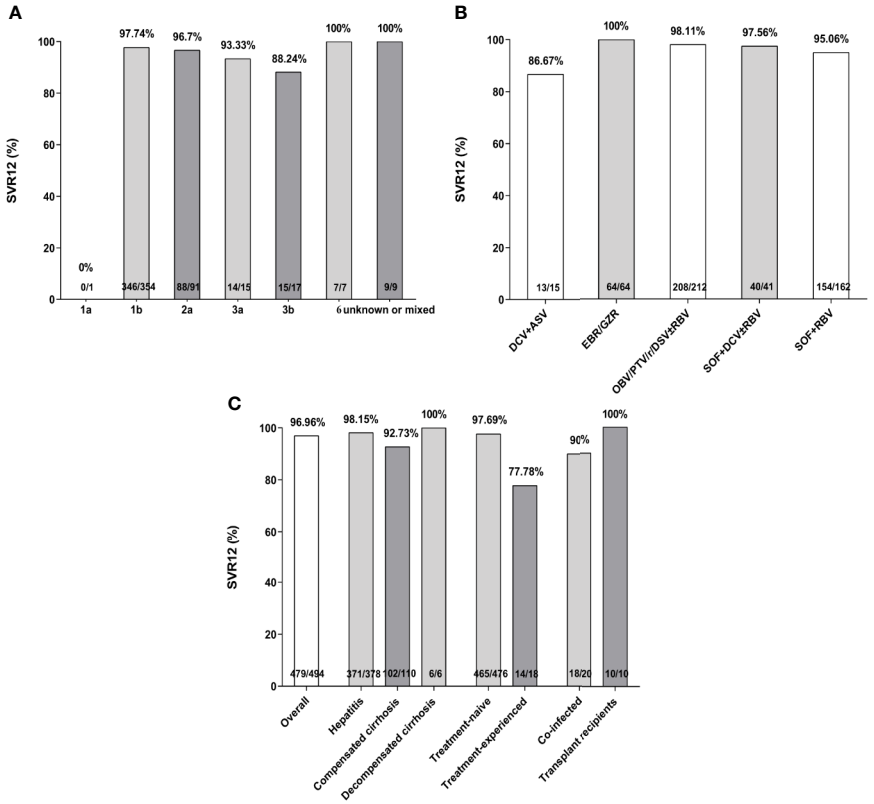

FIGURE 2 | Sustained virological response (SVR) rates (%) according to hepatitis C virus (HCV) genotypes **(A)**, treatment regimens **(B)**, treatment history, liver stage, and specific sub-populations **(C).** SVR, sustained virologic response; SOF, sofosbuvir; RBV, ribavirin; DCV, daclatasvir; EBR/GZR, elbasvir/grazoprevir; OBV/PTV/r/DSV, ombitasvir/paritaprevir/ritonavir and dasabuvir; ASV, asunaprevir.

DISCUSSION

In this retrospective single-center real-life study we assessed the outcome of HCV treatment with available brand DAAs from 2018 to 2019. Real-world data of HCV treatments are essential, especially to verify their efficacy and safety in daily practice outside the range of randomized controlled trials (Flisiak et al., 2017). Because of their high costs in China, brand DAAs are not easily accessible. Hence, few real-world data concerning their efficacy and safety were reported (Zeng et al., 2017; Hu et al., 2018). Moreover, many of these reports used generic DAA drugs, so the results may not represent the real outcomes. This could be an essential issue regarding HCV treatment, particularly in China which has the world's largest HCV-infected population based on the estimated prevalence (Wei and Lok, 2014).

Overall SVR rates higher than 95% were observed across the majority of participants, except those having (1) compensated cirrhosis, (2) prior treatment experience, (3) GT1a and GT3 HCV infection, and (4) treatment with DCV + ASV. With the exception of SVR results from GT1a HCV-infected patients, our results were in accordance with previously published real-world data (Hong et al., 2018; Suzuki et al., 2018; Lobato et al., 2019). The lower than expected SVR rates in GT1a was probably due to the small sample size (only one patient). The patient had cirrhosis and was previously

TABLE 3 | Factors associated with SVR12.

Variables	Unadjusted		Adjusted	
	Univariate OR (95%CI)	*p* value	Multivariate OR (95%CI)	*p* value
Age (years)	1.012 (0.972,1.054)	0.5551	0.967 (0.912,1.024)	0.249
Male	1.248 (0.445,3.495)	0.6737	1.271 (0.276,5.852)	0.7586
HCV RNA (Log_{10} IU/ml)	1.296 (0.672,2.499)	0.4397	1.563 (0.627,3.897)	0.338
Liver stiffness measurement (LSM) (kPa)	1.043 (1.006,1.082)	0.0221	1.041 (0.990,1.093)	0.1156
History of HCC	3.723 (0.441,31.425)	0.2272	<0.001 (<0.001, >999.999)	0.9805
Bilirubin (μmol/L)	1.000 (0.962,1.039)	0.9886	0.975 (0.869,1.094)	0.6676
ALT (U/L)	0.997 (0.985,1.009)	0.6293	0.975 (0.937,1.014)	0.1985
AST (U/L)	1.002 (0.989,1.015)	0.7618	1.006 (0.969,1.046)	0.7429
AFP (ng/ml)	1.002 (1.000,1.003)	0.0303	1.001 (0.999,1.004)	0.3683
Regimens				
SOF+RBV	Ref	Ref	Ref	Ref
SOF+DCV ± RBV	0.481 (0.058,3.961)	0.9615	0.950 (0.080,11.255)	0.9673
EBR/GZR	<0.001 (<0.001, >999.999)	0.9482	<0.001 (<0.001, >999.999)	0.9647
OBV/PTV/r/DSV ± RBV	0.370 (0.109,1.252)	0.9675	0.486 (0.089,2.654)	0.4046
ASV+DCV	2.962 (0.569,15.415)	0.9202	6.595 (0.724,60.036)	0.0941
Platelets (10^9/L)	0.997 (0.989,1.004)	0.4019	0.997 (0.985,1.009)	0.6393

SVR, sustained virologic response; OR, odds ratio; CI, confidence intervals; HCV, hepatitis C virus; IFN, interferon; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; SOF, sofosbuvir; RBV, ribavirin; DCV, daclatasvir; EBR/GZR, elbasvir/grazoprevir; OBV/PTV/r/DSV, ombitasvir/paritaprevir/ritonavir and dasabuvir; ASV, asunaprevir.

TABLE 4 | Characteristics of the reported adverse events.

Adverse events	Total (n = 494)	SOF + RBV (n = 162)	SOF/DCV ± RBV (n = 41)	OBV/PTV/r/DSV ± RBV (n = 212)	EBR/GZR (n = 64)	DCV + ASV (n = 15)
Fatigue	47 (9.5)	15 (9.3)	8 (19.5)	21 (9.9)	2 (3.1)	1 (6.7)
Headache	14 (2.8)	4 (2.5)	2 (4.9)	8 (3.8)	0 (0)	0 (0)
Dizziness	29 (5.9)	7 (4.3)	3 (7.3)	13 (6.1)	5 (7.8)	1 (6.7)
Insomnia	14 (2.8)	5 (3.1)	4 (9.8)	2 (4.7)	2 (3.1)	1 (6.7)
Diarrhea	3 (0.6)	1 (0.6)	0 (0)	1 (0.5)	1 (1.6)	0 (0)
Nausea	11 (2.2)	2 (1.2)	1 (2.4)	5 (2.4)	2 (3.1)	1 (6.7)
Vomiting	11 (2.2)	0 (0)	1 (2.4)	7 (3.3)	3 (4.7)	0 (0)
Anemia	37 (7.5)	21 (12.9)	5 (12.2)	11 (5.2)	0 (0)	0 (0)
Abnormal liver function	24 (4.9)	8 (4.9)	3 (7.3)	10 (4.7)	1 (1.6)	2 (13.3)

Data expressed as sample size and proportion (%).
SOF, sofosbuvir; RBV, ribavirin; DCV, daclatasvir; EBR/GZR, elbasvir/grazoprevir; OBV/PTV/r/DSV, ombitasvir/paritaprevir/ritonavir and dasabuvir; ASV, asunaprevir.

treated with EBR/GZR. Due to the distribution of HCV GTs in Asia (Ji et al., 2018) and China (Chen et al., 2017), HCV GT1a is relatively unusual. Therefore, our results were not sufficient for a meaningful estimation of SVR in HCV GT1a group.

Until the advent of the recently produced DAA (SOF/VEL, glecaprevir/pibrentasvir, SOF/VEL/voxilaprevir), genotype 3 was considered as difficult to cure (Nelson et al., 2015). In fact, many DAAs are less effective on this genotype in general and particularly on its subtype 3b (McPhee, 2019). Our data corroborate previous observations as genotype 3b patients had the lowest SVR12 rates if GT1a result is not considered. Most GT3 patients in our study were treated with SOF + RBV or SOF + DCV + RBV for 24 weeks. Our results are consistent with the findings of a phase 3 clinical trial in which SOF + RBV was administered during 24 weeks to treat Chinese patients with GT3 HCV infection (Huang et al., 2019). The investigators also noted that the presence of cirrhosis lowered SVR rates and the patient who relapsed had genotype 3b infection and cirrhosis. SVR12 rate was relatively low in patients receiving DCV + ASV, which was similar to previous real-world results (Hong et al., 2018; Ji et al., 2018; Itokawa et al., 2019). In our case, this observation was probably due to the very low power of the regimen characterized by its small sample size.

Moreover, the SVR12 rate of decompensated cirrhotic patients (6/6, 100%) was higher than compensated cirrhotic patients (102/110, 92.73%). This finding appears to be driven by either the small sample size, the extended 24-week regimen, or the addition of RBV. Notably, only about one-fifth of our cohort (22%) had cirrhosis, which was most likely due to the favorable reimbursement policy for DAAs therapy in Tianjin, China.

Our study, similarly to previous studies, demonstrated the efficacy of DAAs in the treatment of several sub-populations of HCV-infected individuals (HBV or HIV co-infections and solid organ transplant recipients) (Li et al., 2018; Zhang et al., 2019). Therefore, we suggest that HCV eradication with DAA regimens should not be withheld in these populations as all had excellent results.

Potential drug-drug interactions (DDIs) during DAA therapy should be considered especially when analyzing DAAs treatment response in patients with HIV or HCV co-infection or immunosuppression after solid organ transplantation. In our study, confirmation of DDIs before using DAAs was

recommended. Subsequently, no clinically relevant DDIs was reported which implies that these five DAA regimens are safe even when they are co-administered with other drugs. However, more information are needed to confirm our statements and precautions should be taken.

This study has several limitations. Firstly, it was conducted in a restricted population. Therefore, information concerning the prevalence and distribution of viral and host factors that influence therapeutic outcomes are limited. Secondarily, DAA regimens only concerned brand agents covered by the Tianjin medical insurance programs. In regards to this, our findings cannot be generalized to other types of DAAs. Moreover, baseline NS5A/NS5B resistance-associated substitutions (RAS) testing was not performed. Having them tested would help to reduce a gap encountered in the literature. Actually, it is well-known that some of them, especially those related to NS5A region, were associated to lower response to therapy (Iio et al., 2017). However, some studies have reported that the prevalence of baseline RAS in Chinese HCV patients was relatively low and probably has not affected the SVR results (Wei et al., 2018a; Wei et al., 2018b). Besides, at the end of our data collection, there were a high number of patients (n = 120) who finished DAAs therapy but had not completed the 12-week post-treatment follow-up, their therapeutic effect (virological response) were not available and the current results should be further verified. The final limitation stands in the fact that, as the study describes real-world treatment outcome data, no control group of HCV-infected patients was included.

In summary, our study represents one of the largest cohorts of Chinese patients treated with various brand DAAs regimen available in a real-world setting. The overall SVR12 rates were comparable with that of international clinical trials, and the treatment was safe and well-tolerated. Liver stiffness measurement and AFP were predictors of not achieving SVR12. Future validation studies with a larger number of cases are required. Meanwhile, the current study could represent an important evidence leading to improvement of future strategies regarding the management and the use of DAAs in China.

DATA AVAILABILITY STATEMENT

The datasets analyzed in this article are not publicly available. The raw data required to support the findings of this study cannot be shared at this time as the data also forms part of an ongoing study. Requests to access the datasets should be directed to xiahuan1009@163.com.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients. The study was approved by the Human Medical Ethics Committee of Tianjin Second People's Hospital.

AUTHOR CONTRIBUTIONS

HX and CL contributed equally as co-first authors. PM contributed to the design of this study. HX, YH, YWu, ZY, PM, and CL contributed to data collection. HX, YWa, SZ, and PM analyzed the data and wrote the article. All authors read and approved the final manuscript.

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REFERENCES

Berg, T., Naumann, U., Stoehr, A., Sick, C., John, C., Teuber, G., et al. (2019). Real-world effectiveness and safety of glecaprevir/pibrentasvir for the treatment of chronic hepatitis C infection: data from the German Hepatitis C-Registry. *Aliment Pharmacol. Ther.* 49 (8), 1052–1059. doi: 10.1111/apt.15222

Bian, D. D., Zhou, H. Y., Liu, S., Liu, M., Duan, C., Zhang, J. Y., et al. (2017). Current treatment status and barriers for patients with chronic HCV infection in mainland China: A national multicenter cross-sectional survey in 56 hospitals. *Med. (Baltimore)* 96 (34), e7885. doi: 10.1097/MD.0000000000007885

Bureau, T.M.H.R.A.S.S. (2018). Available: http://hrss.tj.gov.cn/ecdomain/framework/tj/gnnknhmjegifbbodkjajlpafcampibii/innplgdkegifbbodkjajlpafcampibii.do?isfloat=1&fileid=20180328143620370&moduleIDPage=innplgdkegifbbodkjajlpafcampibii&siteIDPage=tj&pageID=gnnknhmjegifbbodkjajlpafcampibii [Accessed April 14, 2020].

Chen, Y., Yu, C., Yin, X., Guo, X., Wu, S., and Hou, J. (2017). Hepatitis C virus genotypes and subtypes circulating in Mainland China. *Emerg. Microbes Infect.* 6 (11), e95. doi: 10.1038/emi.2017.77

European Association for the Study of the Liver, Electronic address, e.e.e. and European Association for the Study of the, L (2018). EASL Recommendations on Treatment of Hepatitis C 2018. *J. Hepatol.* 69 (2), 461–511. doi: 10.1016/j.jhep.2018.03.026

Feld, J. J., Jacobson, I. M., Hezode, C., Asselah, T., Ruane, P. J., Gruener, N., et al. (2015). Sofosbuvir and Velpatasvir for HCV Genotype 1, 2, 4, 5, and 6 Infection. *N. Engl. J. Med.* 373 (27), 2599–2607. doi: 10.1056/NEJMoa1512610

Flisiak, R., Pogorzelska, J., and Flisiak-Jackiewicz, M. (2017). Hepatitis C: efficacy and safety in real life. *Liver Int.* 37 Suppl 1, 26–32. doi: 10.1111/liv.13293

Forns, X., Lee, S. S., Valdes, J., Lens, S., Ghalib, R., Aguilar, H., et al. (2017). Glecaprevir plus pibrentasvir for chronic hepatitis C virus genotype 1, 2, 4, 5, or 6 infection in adults with compensated cirrhosis (EXPEDITION-1): a single-arm, open-label, multicentre phase 3 trial. *Lancet Infect. Dis.* 17 (10), 1062–1068. doi: 10.1016/S1473-3099(17)30496-6

Health, N.I.o (2017). *Cancer Therapy Evaluation Program CTCAE V5.0*, National Institutes of Health, National Cancer Institute. Available: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf. [Accessed April 14, 2020].

Hong, C. M., Liu, C. H., Su, T. H., Yang, H. C., Chen, P. J., Chen, Y. W., et al. (2018). Real-world effectiveness of direct-acting antiviral agents for chronic hepatitis C in Taiwan: Real-world data. *J. Microbiol. Immunol. Infect*. S1684-1182(18)30403-1. doi: 10.1016/j.jmii.2018.09.005

Hu, C., Yuan, G., Liu, J., Huang, H., Ren, Y., Li, Y., et al. (2018). Sofosbuvir-Based Therapies for Patients with Hepatitis C Virus Infection: Real-World Experience in China. *Can. J. Gastroenterol. Hepatol.* 2018, 3908767. doi: 10.1155/2018/3908767

Huang, R., Rao, H., Xie, Q., Gao, Z., Li, W., Jiang, D., et al. (2019). Comparison of the efficacy of sofosbuvir plus ribavirin in Chinese patients with genotype 3a or 3b HCV infection. *J. Med. Virol.* 91 (7), 1313–1318. doi: 10.1002/jmv.25454

Iio, E., Shimada, N., Abe, H., Atsukawa, M., Yoshizawa, K., Takaguchi, K., et al. (2017). Efficacy of daclatasvir/asunaprevir according to resistance-associated variants in chronic hepatitis C with genotype 1. *J. Gastroenterol.* 52 (1), 94–103. doi: 10.1007/s00535-016-1225-x

Itokawa, N., Atsukawa, M., Tsubota, A., Ikegami, T., Shimada, N., Kato, K., et al. (2019). Efficacy of direct-acting antiviral treatment in patients with compensated liver cirrhosis: A multicenter study. *Hepatol. Res.* 49 (2), 125–135. doi: 10.1111/hepr.13256

Ji, F., Wei, B., Yeo, Y. H., Ogawa, E., Zou, B., Stave, C. D., et al. (2018). Systematic review with meta-analysis: effectiveness and tolerability of interferon-free direct-acting antiviral regimens for chronic hepatitis C genotype 1 in routine clinical practice in Asia. *Aliment Pharmacol. Ther.* 47 (5), 550–562. doi: 10.1111/apt.14507

Kwo, P. Y., Poordad, F., Asatryan, A., Wang, S., Wyles, D. L., Hassanein, T., et al. (2017). Glecaprevir and pibrentasvir yield high response rates in patients with HCV genotype 1-6 without cirrhosis. *J. Hepatol.* 67 (2), 263–271. doi: 10.1016/j.jhep.2017.03.039

Lawitz, E., Poordad, F., Wells, J., Hyland, R. H., Yang, Y., Dvory-Sobol, H., et al. (2017). Sofosbuvir-velpatasvir-voxilaprevir with or without ribavirin in direct-acting antiviral-experienced patients with genotype 1 hepatitis C virus. *Hepatology* 65 (6), 1803–1809. doi: 10.1002/hep.29130

Li, Y., Li, L., Liu, J., Zhang, D. W., Zhao, F., Wang, L., et al. (2018). Tolerable and curable treatment in HIV/HCV co-infected patients using anti-HCV direct antiviral agents: a real-world observation in China. *Hepatol. Int.* 12 (5), 465–473. doi: 10.1007/s12072-018-9891-9

Liu, Z., Yang, Q., Shi, O., Ye, W., Chen, X., and Zhang, T. (2018). The epidemiology of hepatitis B and hepatitis C infections in China from 2004 to 2014: An observational population-based study. *J. Viral. Hepat.* 25 (12), 1543–1554. doi: 10.1111/jvh.12938

Lobato, C. M. O., Codes, L., Silva, G. F., Souza, A. F. M., Coelho, H. S. M., Pedroso, M. L. A., et al (2019). Direct antiviral therapy for treatment of hepatitis C: A real-world study from Brazil. *Ann. Hepatol.* 18 (6), 849–854. doi: 10.1016/j.aohep.2019.08.001

McPhee, F. (2019). Developments in the treatment of HCV genotype 3 infection. *Expert Rev. Anti. Infect. Ther.* 17 (10), 775–785. doi: 10.1080/14787210.2019.1676730

Mera, J., Joshi, K., Thornton, K., Box, T., Scott, J., Sedillo, M., et al. (2019). Retrospective Study Demonstrating High Rates of Sustained Virologic Response After Treatment With Direct-Acting Antivirals Among American Indian/Alaskan Natives. *Open Forum Infect. Dis.* 6 (7), ofz128. doi: 10.1093/ofid/ofz128

Nelson, D. R., Cooper, J. N., Lalezari, J. P., Lawitz, E., Pockros, P. J., Gitlin, N., et al. (2015). All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. *Hepatology* 61 (4), 1127–1135. doi: 10.1002/hep.27726

Omata, M., Kanda, T., Wei, L., Yu, M. L., Chuang, W. L., Ibrahim, A., et al. (2016). APASL consensus statements and recommendation on treatment of hepatitis C. *Hepatol. Int.* 10 (5), 702–726. doi: 10.1007/s12072-016-9717-6

Polaris Observatory, H. C. V. C. (2017). Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. *Lancet Gastroenterol. Hepatol.* 2 (3), 161–176. doi: 10.1016/S2468-1253(16)30181-9

Saxena, V., Khungar, V., Verna, E. C., Levitsky, J., Brown, R. S. Jr., Hassan, M. A., et al. (2017). Safety and efficacy of current direct-acting antiviral regimens in kidney and liver transplant recipients with hepatitis C: Results from the HCV-TARGET study. *Hepatology* 66 (4), 1090–1101. doi: 10.1002/hep.29258

Suzuki, F., Hatanaka, N., Bando, E., Nakamura, K., and Komoto, A. (2018). Safety and effectiveness of daclatasvir and asunaprevir dual therapy in patients with genotype 1 chronic hepatitis C: results from postmarketing surveillance in Japan. *Hepatol. Int.* 12 (3), 244–253. doi: 10.1007/s12072-018-9872-z

Wei, L., and Lok, A. S. (2014). Impact of new hepatitis C treatments in different regions of the world. *Gastroenterology* 1461145-1150 (5), e1141–e1144. doi: 10.1053/j.gastro.2014.03.008

Wei, L., Xie, Q., Hou, J. L., Jia, J., Li, W., Xu, M., et al. (2018a). Sofosbuvir plus ribavirin with or without peginterferon for the treatment of hepatitis C virus: Results from a phase 3b study in China. *J. Gastroenterol. Hepatol.* 33 (6), 1168–1176. doi: 10.1111/jgh.14102

Wei, L., Xie, Q., Hou, J. L., Tang, H., Ning, Q., Cheng, J., et al. (2018b). Ledipasvir/sofosbuvir for treatment-naive and treatment-experienced Chinese patients with genotype 1 HCV: an open-label, phase 3b study. *Hepatol. Int.* 12 (2), 126–132. doi: 10.1007/s12072-018-9856-z

Zeng, Q. L., Xu, G. H., Zhang, J. Y., Li, W., Zhang, D. W., Li, Z. Q., et al. (2017). Generic ledipasvir-sofosbuvir for patients with chronic hepatitis C: A real-life observational study. *J. Hepatol.* 66 (6), 1123–1129. doi: 10.1016/j.jhep.2017.01.025

Zhang, J., Sun, W., Lin, J., Tian, Y., Ma, L., Zhang, L., et al. (2019). Long-term follow-up of HCV infected kidney transplant recipients receiving direct-acting antiviral agents: a single-center experience in China. *BMC Infect. Dis.* 19 (1), 645. doi: 10.1186/s12879-019-4217-7

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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MINI REVIEW
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Available Evidence and Ongoing Clinical Trials of Remdesivir: Could It Be a Promising Therapeutic Option for COVID-19?

Mekonnen Sisay *

Department of Pharmacology and Toxicology, School of Pharmacy, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia

The novel coronavirus strain, severe acute respiratory syndrome coronavirus-2, the causative agent of COVID-19 emerged in Wuhan, China, in December 2019 and is skyrocketing throughout the globe and become a global public health emergency. Despite promising preventive measures being taken, there is no vaccine or drug therapy officially approved to prevent or treat the infection. Everybody is waiting the findings of ongoing clinical trials in various chemical and biological products. This review is specifically aimed to summarize the available evidence and ongoing clinical trials of remdesivir as a potential therapeutic option for COVID-19. Remdesivir is an investigational drug having broad spectrum antiviral activity with its target RNA dependent RNA polymerase. It has not yet been officially approved for Ebola and Coronaviruses. Several studies showed that remdesivir had promising *in vitro* and *in vivo* antiviral activities against SARS-CoV-1 and MERS-CoV strains. On the top of this, it exhibited a promising *in vitro* activity against SARS-CoV-2 strains though there are no published studies that substantiate its activity *in vivo* until the time of this review. There are few phase 3 randomized double-blind placebo controlled trials on the way to investigate the safety and efficacy of remdesivir. Of which, one completed double blind, placebo controlled trial showed that remdesivir showed faster time to clinical improvement in severe COVID-19 patients compared to placebo though not found statistically significant. In addition, two phase 3 randomized open label clinical trials coordinated by Gilead Sciences are being conducted. In addition, WHO Solidarity trial and INSERM DisCoVeRy trials (randomized open labels) were launched recently.

Keywords: SARS-CoV-2, COVID-19, remdesivir, RdRp, GS-5734

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Min Yang,
Anhui Medical University, China

Reviewed by:
Rachel Graham,
University of North Carolina at Chapel Hill, United States
Joao Massud,
Independent Researcher, São Paulo, Brazil

***Correspondence:**
Mekonnen Sisay
mekonnensisay27@yahoo.com

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BACKGROUND

Coronaviruses, belonging to the family Coronaviridae, are positive-sense enveloped RNA viruses that cause infections in humans (Weiss and Leibowitz, 2011; Lim et al., 2016). The family includes four genera (Alphacoronavirus, Betacoronavirus, Deltacoronavirus, and Gammacoronavirus). The genus Betacoronavirus includes severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle

East respiratory syndrome coronavirus (MERS-CoV). Historically, these coronaviruses had got great clinical importance in infecting humans (Kuiken et al., 2003). At present, the novel coronavirus strain, the SARS-CoV-2, the causative agent of COVID-19 emerged in Wuhan, China, in December 2019 (Zhu et al., 2020). Since then, the number of cases and deaths related to this virus have been skyrocketing throughout the world.

As per the World Health Organization (WHO) report, the total number of cases and deaths outside China has overtaken the total number of cases in China (WHO, 2020). WHO has declared COVID-19 worldwide pandemic and global public health emergency with Europe and lately Unites States of America became new epicenters. WHO has recommended several preventive measures including laboratory tests for any suspected cases, quarantining suspects, applying physical distancing, frequent hand washing, and using hand and surface sanitizers to help contain further spread of the pandemic (WHO, 2020). Despite such preventive strategies, there is no vaccine or drug therapy officially approved for prophylaxis or treatment of COVID-19. At present, there are several classes of drugs undergoing clinical trials including RNA polymerase inhibitors (remdesivir and favipiravir), protease inhibitors (lopinavir/ritonavir), aminoquinolines (chloroquine and its hydroxyl derivative), anti-inflammatory agents (corticosteroids, and xiyanping injection), angiotensin converting enzyme type 2 blockers, convalescent plasma, viral RNA antisense technologies, monoclonal antibodies, and Chinese traditional medicines (http://www.chictr.org.cn/index.aspx and https://clinicaltrials.gov/ct2/home).

OVERVIEW OF OTHER FRONTLINE ANTIVIRAL AGENTS UNDER EXTENSIVE CLINICAL INVESTIGATION

Among the above-mentioned classes of antiviral counterparts, aminoquinolines (chloroquine and its hydroxyl derivative) and protease inhibitors (primarily lopinavir/ritonavir) have taken the largest share of clinical trials since the SARS-CoV-2 outbreak. However, their clinical benefit has become full of controversies according to the various research findings.

With regard to the aminoquinolines and their role in COVID-19 therapy, in an open-label non-randomized clinical trial, Gautret et al. reported 100% viral clearance in nasopharyngeal swabs with combination of hydroxychloroquine and azithromycin, 57.1% in hydroxychloroquine group, and 12.5% in standard of care group in cohort of 6 patients after 5 to 6 days follow-up (Gautret et al., 2020). In addition, in study conducted in 62 patients in China, the use of hydroxychloroquine could significantly shorten the time to clinical recovery and promote the absorption of pneumonia in randomized open label clinical trial (Chen Z. et al., 2020). In contrary to this, a recent study from China in individuals with COVID-19 found no difference in the rate of virologic clearance at 7 days with or without 5 days of hydroxychloroquine, and no difference in clinical outcomes (Chen J. et al., 2020) indicating the absence of evidence of a strong antiviral activity (rapid viral clearance) or clinical benefit of this combination for severe COVID-19 patients. Another randomized open label clinical trial posted on MedRxiv (preprint) reported that the overall 28-day negative conversion rate and symptoms alleviation rate in hydroxychloroquine plus standard of care group was not different from standard of care group (Tang et al., 2020). Apart from this, the safety issues of aminoquinolines should also be emphasized. The cardiovascular and retinal toxicities may also limit the usefulness of these agents if there is hope from ongoing randomized, blinded and placebo controlled trials. For example, the hydroxychloroquine and azithromycin have shown to prolong QT-interval resulting torsades de pointes (a form of polymorphic ventricular tachycardia) and sudden cardiac death (Chorin et al., 2020).

A randomized, open label clinical trial conducted on protease inhibitors (lopinavir/ritonavir) indicated that treatment with lopinavir/ritonavir did not show statistically significant difference in the time to clinical improvement and mortality censored at day 28 though a secondary outcomes measures were found promising in lopinavir/ritonavir group (Cao et al., 2020). This trial was initiated in severe COVID-19 patients lately and lacks blinding and well established placebo.

Remdesivir is one of the frontline medications being used as expanded access and is under extensive clinical investigation. Hereafter, this review aims to address the viral polymerase inhibitor remdesivir as a potential therapeutic option for COVID-19.

REMDESIVIR: CHEMISTRY AND MECHANISM OF ACTION

Remdesivir (GS-5734^{TM}) is a phosphoramidate prodrug of a Pyrrolo[2,1-*f*][triazin-4-amino] adenine **C**-nucleoside having broad spectrum antiviral activity (**Figure 1**). Remdesivir is metabolized into its active form, GS-441524, that interferes with viral RNA dependent RNA polymerase (RdRp) enzyme thereby it evades proofreading by viral exonuclease, and arrests RNA synthesis. This drug has shown potent inhibitory activity against RdRp with intact proof reading and with low level of resistance to target mutations (Agostini et al., 2018). It is supported in the study conducted by Gordon et al. who demonstrated RdRp is, indeed, the target of remdesivir in MERS-CoV strains (Gordon et al., 2020). Studies based on the molecular dynamics simulation and free energy perturbation methods clearly indicated SARS-CoV-2 RdRp as a target of remdesivir (Zhang and Zhou, 2020). Though, it has not been officially approved for Ebola and Coronaviruses yet (Siegel et al., 2017), Gilead Sciences is working closely with organizations and health authorities to respond to the COVID-19 outbreak through synthesizing and providing this investigational drug (Gilead sciences, 2020a).

EVIDENCE ON ITS *IN VITRO* ACTIVITY

Agostini et al. demonstrated that remdesivir can potently inhibit coronaviruses such as SARS-CoV-1 and MERS-CoV *in vitro* (Agostini et al., 2018). Remdesivir can inhibit SARS-CoV-1 and

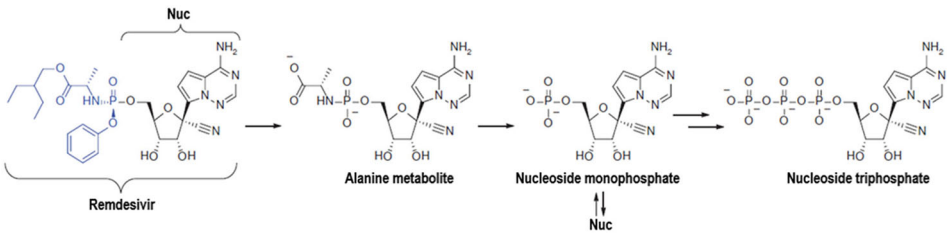

FIGURE 1 | Structure and metabolic conversion of remdesivir.

MERS-CoV replication in several *in vitro* systems, including primary human airway epithelial cell cultures (Sheahan et al., 2017). In research conducted by Sheahan et al, remdesivir showed superior antiviral activity to lopinavir/ritonavir against MERS-COV *in vitro* (Sheahan et al., 2020). In MERS-COV nonstructural proteins (nsp5, nsp7, nsp8, and nsp12) of insect cell lines, remdesivir showed potent inhibitory activity against nsp12(RdRp) *in vitro* (Gordon et al., 2020). Yethindra et al. demonstrated that remdesivir showed strong inhibition against SARS-CoV and MERS-CoV in human air way epithelial cells, at early stages in replication process *via* inhibiting viral RNA synthesis (Yethindra, 2020). On the top of these, remdesivir has shown promising results in clinical control of SARS-CoV-2 pneumonia *in-vitro* in human liver cancer cell lines (Wang M. et al., 2020). Beyond Beta-CoVs, remdesivir has shown potent inhibition of human endemic and zoonotic Delta-CoVs with highly divergent RdRp in human hematoma (huh7) cell lines (Brown et al., 2019).

EVIDENCE ON *IN VIVO* ANTIVIRAL ACTIVITY

In a mouse model of SARS-CoV-1, prophylactic and therapeutic (at early stage) administration of remdesivir significantly reduced pulmonary viral load and improved respiratory function and other clinical signs of the disease (Sheahan et al., 2017). Likewise, both prophylactic and therapeutic remdesivir has shown improvement on the pulmonary function and reduced lung viral loads and severe lung pathology in MERS-COV strains in mice model (Sheahan et al., 2020). In the rhesus macaque model of MERS-CoV infection, remdesivir reduced virus replication, the severity of the disease, and lung damage when administered in animals infected with MERS-CoV (de Wit et al., 2020). Despite having an *in-vitro* antiviral activity against SARS-CoV-2, there are no published studies justifying the activity of remdesivir in animal models of SARS-CoV-2 *in vivo* until the time of this review.

CASE REPORT

According to the paper published at the New England Journal of Medicine on 05 March, 2020, it had been suggested that remdesivir might be a potential therapeutic option for the therapy of COVID-19 patients. In the report, remdisivir intravenous infusion (compassionate use) was started on day 7 in COVID-19 patient. During the treatemnt, no adverse events were observed in association with the IV infusion. The patient's clinical condition improved. The bilateral lower-lobe rales apeared initially were no longer present. His appetite improved, became afebrile and asymptomatic except intermittent dry cough and rhinorrhea (Holshue et al., 2020). However, this is a single patient report and is too infant to conclude its efficacy and disentangle the true effect size of this drug because of the chance of recovery from this disease without treatment(s). Hence, it is imperative to have adequate, well controlled, randomized, and blinded clinical trials in large cohorts of patients to justify its clinical utility in real settings.

ONGOING CLINICAL TRIALS AND FUTURE PROSPECTS

As summarized in **Table 1**, Gilead Sciences has initiated two phase 3 randomized, open label clinical trials comprising approximately 1,000 COVID-19 patients. In the first trial, 400 patients with severe COVID-19 cases were enrolled to evaluate the safety and efficacy of remdesivir on 5 (Arm 1) and 10 days (Arm 2) regimens with standard of care in both arms without comparator (Gilead-Sciences, 2020b). In the second trial, 600 patients with moderate COVID-19 cases were enrolled to evaluate the safety and efficacy of the same dosage regimen of remdesivir in addition to the standard of care and with standrad of care alone as active comparator (Gilead-Sciences, 2020c).

With the coordination of China-Japan Friendship Hospital, two phase 3 randomized, double blind, placebo controlled clinical trials were intiated in China to evaluate the safety and efficacy of remdesivir paralleled to palcebo therapy.The first phase 3 trial has already involved 308 hospitalized adult patients with mild to moderate cases of COVID-19 (Cao, 2020a). The patients were randomized to intervention arms of either remdesivir 10 days regimen or placebo that matched to remdesivir. The primary end point of this trial is set to determine the time to clinical recovery (TTCR) within 28 days. Another phase 3 randomized, double-blind, placebo-controlled trial is evaluating the safety and efficacy of 10 days of remdesivir regimen in 453 hospitalized adult patients with severe COVID-19 compared to placebo matched to remdesivir in

TABLE 1 | Ongoing clinical trials registered under United States National Library of Medicine clinical trials registry and addressing the safety and efficacy of remdesivir (GS-5734™) as a potential therapeutic option for COVID 19.

Clinical trial identifier	Study design	Estimated enrollment	Phase of the study	Conditions	Interventions		Primary outcome measures	Recruitment status
					Experimental arm	Comparator (control) arm		
NCT04292899 (Gilead-Sciences, 2020b)	Randomized, open label clinical trial	400	Phase 3	Severe COVID 19	**Arm 1:** Remdesivir IV infusion for 5 Days + standardized care **Arm 2**: Remdesivir IV infusion 10 Days	**Active:** No placebo	Composite outcome measure (proportion of participants with normalization of fever and oxygen saturation through day 14)	Recruiting
NCT04292730 (Gilead-Sciences, 2020c)	Randomized, open label clinical trial	600	Phase 3	Moderate COVID 19	**Arm 1:** Remdesivir IV infusion for 5 Days + standardized care **Arm 2:** Remdesivir IV infusion 10 Days	**Arm 3:** Active (Standard of Care)	Proportion of participants discharged by day 14	Recruiting
NCT04252664 (Cao, 2020a)	Randomized, Double-blind, Placebo-controlled clinical trial	308	Phase 3	Mild and Moderate COVID 19	**Arm 1:** Remdesivir IV for 10 Days (200 mg loading dose for day 1 followed by 100 mg IV for 9 days)	**Arm 2**: Placebo that match remdesivir in dose and duration	Time to clinical recovery (TTCR) Upto 28 days	Recruiting
NCT04257656 (Cao, 2020b)	Randomized, Double-blind, Placebo-controlled clinical trial	453	Phase 3	Severe COVID 19	**Arm 1:** Remdesivir IV for 10 Days (200 mg loading dose for day 1 followed by 100 mg IV for 9 days)	**Arm 2:** Placebo that match remdesivir in dose and duration	Time to Clinical Improvement (TTCI) [Censored at Day 28]	Completed
NCT04280705 (NIAID, 2020)	Adaptive, Randomized, double Blind Controlled Trial	394 (1:1)	Phase 3	Hospitalized patients with COVID 19 (no specific severity)	**Arm 1:** 200 mg RDV IV for day 1 followed by 100 mg IV QD for 9 days	**Arm 2**: Placebo that match remdesivir in dose and duration	Percentage of subjects reporting each severity rating on the 7-point ordinal scale within 15 days	Recruiting
NCT04302766 (US-AMRDC, 2020)	Expanded access	General (Intermediate-size Population)	NA	Any COVID 19 case	Not stated	Not stated	NA	Available
NCT04315948 **(DisCoVeRy trial**) (INSERM, 2020)	Adaptive, Randomized, Open label clinical Trial	3200	Phase 3	COVID-19 in hospitalized adults	**Arm 1:** 200 mg RDV IV for day 1 followed by 100 mg IV QD for 9 days **Arm 2:** Lopinavir/ritonavir (400 mg/100 mg) tablet BID for 14 days **Arm 3:** Lopinavir/ritonavir (400 mg/100 mg) tablet BID for 14 days plus Interferon ß-1a 44 ug subcutaneously in total of three doses (Day 1, 3 and 6)	**Arm 4:** (Active) Standard of care	Percentage of subjects reporting each severity rating on a 7-point ordinal scale within 15 days	Not yet recruiting

dose and duration. The primary outcome measure in this trial is the time to clinical improvement (TTCI) censored at day 28 (Cao, 2020b) (**Table 1**). At the time of this revision, the finding of the second trial was published on Lancet. Though not statistically significant, patients receiving remdesivir had a numerically faster time to clinical improvement than those receiving placebo among patients with symptom duration of 10 days or less (Hazard ratio 1.52 [0.95–2.43]). In addition, remdesivir did not show statistically significant clinical benefits (Wang Y. et al., 2020). However, the numerical reduction in time to clinical improvement in those treated earlier requires confirmation in the larger cohort of patients and in the remaining trials

With the coordination of the U.S. National Institute of Allergy and Infectious Diseases (NIAID), Another phase 3 adaptive, randomized, double blind, placebo controlled clinical trial enrolled 394 hospitalized patients with COVID-19 to assess the safety and efficacy of remdesivir. In this trial, patients were randomized to either placebo or 10 days therapy with remdesivir (200 mg remdesivir IV loading dose for day 1 followed by 100 mg IV daily maintained for 9 days, total of 10 days therapy). The primary indicator in this trial is the percentage of patients reporting each severity rating on the 7-point ordinal scale within 15 days (NIAID, 2020) (**Table 1**).

With the sponsor and coordination of U.S. Army Medical Research and Development Command (AMRDC), remdesivir is also provided as expanded access (compassionate use) (US-AMRDC, 2020) through emergency investigational new drug applications. The term expanded access (compassionate use) is a potential pathway in which a patient with an immediately life-threatening condition or disease gain access to an investigational medical product for treatment of patients outside of clinical trials when no comparable or satisfactory alternative therapy options are available (FDA, 2020). To this end, remdesivir synthesized and developed by Gilead Sciences is readily available for compassionate use for COVID-19 patients (Coppock, 2020) (**Table 1**). Latest observational study published on The New England Journal of Medicine revealed that severe Covid-19 patients treated with compassionate-use of remdesivir showed clinical improvement in 68% cases (Grein et al., 2020).

On the top of this, WHO has initiated Solidarity trial at global level to evaluate the safety and efficacy of remdesivir in one of the interventional arms (Branswell, 2020). Likewise, National Institute of Health and Medical Research (INSERM) of France has planned to initiate DisCoVeRy trial (INSERM, 2020). The trial protocol has already been registered on 20 March, 2020 and available at https://clinicaltrials.gov/ct2/show/NCT04315948. The recruiting of participants has not been started but estimated to enroll 3,200 patients in 4 arms in which remdesivir alone is to be provided in usual dosage regimen in one of the interventional arms.

CONCLUSION

Despite the promising effects of remdesivir against previous beta-coronaviruses as well as the current novel coronaviruses *in vitro*, there is no published *in vivo* study that substantiates the *in vitro* activities against this global public health threat. A case report and observational studies are not sufficient to generate evidenced-based medicine on the clinical use of remdesivir for this pandemic. A double blind, placebo controlled randomized clinical trial showed that remdesivir did not have statistically significant clinical benefit in reducing the time to clinical improvement in severe COVID-19 patients compared to placebo. Though remdesivir is readily available for compassionate use in many countries, it is imperative to wait the remaining ongoing clinical trials to justify its clinical utility on larger cohort of COVID-19 patients.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

Agostini, M. L., Andres, E. L., Sims, A. C., Graham, R. L., Sheahan, T. P., Lu, X., et al. (2018). Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. *MBio* 9, e`1–00218. doi: 10.1128/mBio.00221-18

Branswell, H. (2020). *WHO to launch multinational trial to jumpstart search for coronavirus drugs* (World Health Organization). Available at: https://www.statnews.com/2020/03/18/who-to-launch-multinational-trial-to-jumpstart-search-for-coronavirus-drugs/. Accessed on 20 March, 2020.

Brown, A. J., Won, J. J., Graham, R. L., Dinnon III, K. H., Sims, A. C., Feng, J. Y., et al. (2019). Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antiviral Res.* 169, 104541. doi: 10.1016/j.antiviral.2019.104541

Cao, B., Wang, Y., Wen, D., Liu, W., Jingli, W., Fan, G., et al. (2020). A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. *New Engl. J. Med.* doi: 10.1056/NEJMoa2001282. 2020.

Cao, B. (2020a). *Mild/Moderate 2019-nCoV Remdesivir RCT*, Available at: https://clinicaltrials.gov/ct2/show/NCT04252664 Accessed on 20, March, 2020.

Cao, B. (2020b). *Severe 2019-nCoV Remdesivir RCT*, avaialble at: https://clinicaltrials.gov/ct2/show/NCT04257656. Accessed on 20 March, 2020.

Chen, J., Liu, D., Lui, L., Liu, P., Xu, Q., Xia, L., et al. (2020). A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 COVID-19). *J. Zhejiang Univ. Sci.* 49, 215–219. doi: 10.3785/j.issn.1008-9292.2020.03.03

Chen, Z., Hu, J., Zhang, Z., Jiang, S., Han, S., Yan, D., et al. (2020). Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial. *medRxiv*. doi: 10.1101/2020.03.22.20040758

Chorin, E., Dai, M., Shulman, E., Wadhwani, L., Bar-Cohen, R., Barbhaiya, C., et al. (2020). The QT interval in patients with COVID-19 treated with hydroxychloroquine and azithromycin. *Nat. Med.* doi: 10.1038/s41591-020-0888-2

Coppock, K. (2020). *FDA Announces Two Drugs Approved for 'Compassionate Use' in Treating COVID-19*. (Pharmacy Times). Available at: https://www.pharmacytimes.com/news/fda-announces-two-drugs-approved-for-compassionate-use-in-treating-covid-19. Accessed on 20 March, 2020.

de Wit, E., Feldmann, F., Cronin, J., Jordan, R., Okumura, A., Thomas, T., et al. (2020). Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. *Proc. Natl. Acad. Sci.* 117, 6771–6776. doi: 10.1073/pnas.1922083117 PNAS

FDA. (2020). *United States Food and Drug Administration*, Expanded Access. (U.S. FDA). Available at: https://www.fda.gov/news-events/public-health-focus/expanded-access. Accessed on 20 March, 2020.

Gautret, P., Lagier, J. C., Parola, P., Hoang, V. T., Meddeb, L., Mailhe, M., et al. (2020). Hydroxychloroquine and azithromycin as atreatment of COVID-19: results of an open-label non-randomised clinical trial. *Int. J. Antimicrob. Agents*. doi: 10.1016/j.ijantimicag.2020.105949

Gilead sciences. (2020a). *Gilead Sciences Update On The Company"s Ongoing Response To COVID-19*. (GILEAD). Available at: https://www.gilead.com/purpose/advancing-global-health/covid-19. Accessed on 20 March, 2020.

Gilead-Sciences. (2020b). *Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734™) in Participants With Severe Coronavirus Disease (COVID-19)*, Available at: https://clinicaltrials.gov/ct2/show/NCT04292899. Accessed on 20 March, 2020.

Gilead-Sciences. (2020c). *Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734™) in Participants With Moderate Coronavirus Disease (COVID-19) Compared to Standard of Care Treatment*, Available at: https://clinicaltrials.gov/ct2/show/NCT04292730 Accessed on 20 March, 2020.

Gordon, C. J., Tchesnokov, E. P., Feng, J. Y., Porter, D. P., and Gotte, M. (2020). The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J. Biol. Chem.* AC120. 013056. doi: 10.1074/jbc.AC120.013056

Grein, J., Ohmagari, N., Shin, D., Diaz, G., Asperges, E., Castagna, A., et al. (2020). Compassionate Use of Remdesivir for Patients with Severe Covid-19. *New Engl. J. Med*. doi: 10.1056/NEJMoa2007016

Holshue, M. L., DeBolt, C., Lindquist, S., Lofy, K. H., Wiesman, J., Bruce, H., et al. (2020). First case of 2019 novel coronavirus in the United States. *New Engl. J. Med*. doi: 10.1056/NEJMoa2001191

INSERM. (2020). *Trial of Treatments for COVID-19 in Hospitalized Adults (DisCoVeRy)*, Available at: https://clinicaltrials.gov/ct2/show/NCT04315948. Accessed on 20 March, 2020.

Kuiken, T., Fouchier, R. A., Schutten, M., Rimmelzwaan, G. F., Van Amerongen, G., van Riel, D., et al. (2003). Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* 362, 263–270. doi: 10.1016/S0140-6736(03)13967-0

Lim, Y. X., Ng, Y. L., Tam, J. P., and Liu, D. X. (2016). Human coronaviruses: a review of virus host interactions. *Diseases* 4, 26. doi: 10.3390/diseases4030026

NIAID. (2020). *Adaptive COVID-19 Treatment Trial*, Available at: https://clinicaltrials.gov/ct2/show/NCT04280705. Accessed on 20 March, 2020.

Sheahan, T. P., Sims, A. C., Graham, R. L., Menachery, V. D., Gralinski, L. E., Case, J. B., et al. (2017). Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Sci. Trans. Med.* 9. doi: 10.1126/scitranslmed.aal3653

Sheahan, T. P., Sims, A. C., Leist, S. R., Schafer, A., Won, J., Brown, A. J., et al. (2020). Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat. Commun.* 11, 1–14. doi: 10.1038/s41467-019-13940-6

Siegel, D., Hui, H. C., Doerffler, E., Clarke, M. O., Chun, K., Zhang, L., et al. (2017). Discovery and Synthesis of a Phosphoramidate Prodrug of a Pyrrolo [2, 1-f] [triazin-4-amino] Adenine C-Nucleoside (GS-5734) for the Treatment of Ebola and Emerging Viruses. *J. Med. Chem.* 60, 1648–1661. doi: 10.1021/acs.jmedchem.6b01594

Tang, W., Cao, Z., Han, M., Wang, Z., Chen, J., Sun, W., et al. (2020). Hydroxychloroquine in patients with COVID-19: an open-label, randomized, controlled trial. *medRxiv*. doi: 10.1101/2020.04.10.20060558

US-AMRDC. (2020). *Expanded Access Remdesivir (RDV; GS-5734™)*>, available at: https://clinicaltrials.gov/ct2/show/NCT04302766. Accessed on 20 March, 2020.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., et al. (2020). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus, (2019-nCoV) in vitro. *Cell Res.* 30, 269–271. doi: 10.1038/s41422-020-0282-0

Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y., et al. (2020). Remdesivir in adults with severe COVID-19: a randomised,double-blind, placebo-controlled, multicentre trial. *Lancet*. doi: 10.1016/S0140-6736(20)31022-9

Weiss, S. R., and Leibowitz, J. L. (2011). Coronavirus pathogenesis *Adv. Virus Res.* 81, 85–164. doi: 10.1016/B978-0-12-385885-6.00009-2.

WHO. (2020). *WHO. World Health Organization situation report*. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200316-sitrep-56-covid-19.pdf?sfvrsn=9fda7db2_2. Accessed on 20 March, 2020.

Yethindra, V. (2020). Role of GS-5734 (Remdesivir) in inhibiting SARS-CoV and MERS-CoV: The expected role of GS-5734 (Remdesivir) in COVID-19 (2019-nCoV)-VYTR hypothesis. *Int. J. Res. Pharm. Sci.* 11, 1–6. doi: 10.26452/ijrps.v11iSPL1.1973

Zhang, L. , and Zhou, R. (2020). Binding Mechanism of Remdesivir to SARS-CoV-2 RNA Dependent RNA Polymerase. *Preprints*. 202003026. doi: 10.20944/preprints202003.0267.v1

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., et al. (2020). A Novel Coronavirus from Patients with Pneumonia in China 2019. *New Engl. J. Med.* doi: 10.1056/NEJMoa2001017

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Efficacy and Safety of Recombinant Human Thrombopoietin on Sepsis Patients With Thrombocytopenia: A Systematic Review and Meta-Analysis

Jin Zhang[1,2], *Zongqing Lu*[1,2], *Wenyan Xiao*[1,2], *Tianfeng Hua*[1,2], *Yao Zheng*[1,2] *and Min Yang*[1,2]*

[1] The Second Department of Intensive Care Unit, the Second Affiliated Hospital of Anhui Medical University, Hefei, China, [2] The Laboratory of Cardiopulmonary Resuscitation and Critical Care Medicine, the Second Affiliated Hospital of Anhui Medical University, Hefei, China

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***Correspondence:**
Min Yang
512130761@qq.com

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Background: The efficacy and safety of the administration of recombinant human thrombopoietin (rhTPO) in sepsis patients with thrombocytopenia were still inconclusive.

Objectives: To investigate whether rhTPO is a benefit for sepsis patients with thrombocytopenia.

Methods: PubMed, Cochrane library, Embase, China National Knowledge Infrastructure, and Wanfang Database were electronically searched to the randomized controlled trials (RCTs) from inception to March 4, 2020. The primary outcome was the level of platelet (PLT) on the 7th day of treatment, and secondary outcomes were 28-d mortality, the level of coagulation indicators, hepatic and renal function indicators, blood transfusion, and length of intensive care unit (ICU) stay.

Results: Ten RCTs involving 681 patients were included. For compared with conventional antibiotic therapy, rhTPO could significantly increase platelet counts (PCs) [standardized mean difference (SMD), 2.61; 95% confidence interval (CI), 1.28–3.94; $P < 0.001$], decreased 28-d mortality [relative risk (RR), 0.66; 95%CI, 0.46–0.97; P=0.03], transfusion volume of blood products and length of ICU stay. Additionally, for compared with conventional antibiotic therapy combined with intravenous immunoglobulin, the pooled results shown that rhTPO also associated with an improvement of PCs on 7th of treatment (SMD, 0.86; 95%CI, 0.54–1.17; $P < 0.001$), and a reduced transfusion volume of blood products. However, there were no differences in 28-d mortality and the length of ICU stay.

Conclusions: Current evidence shown that rhTPO could increase PCs on 7th day of treatment and reduce the transfusion volume of blood products in sepsis-related

thrombocytopenia during hospitalization. The conclusions are needed to be verified indeed by more multicenter RCTs due to the limitation of the included studies.

Keywords: sepsis, thrombocytopenia, recombinant human thrombopoietin (rhTPO), platelet, sepsis-related thrombocytopenia

INTRODUCTION

Although after decades of diagnosis, care, and treatment have improved, sepsis remains a threat to current public health and places a heavy burden on the global economy. Epidemiological studies suggested that the global incidence of sepsis was about 31.5 million and the mortality rate was 16.8% per year (Fleischmann et al., 2016). Thrombocytopenia is a common complication in sepsis patients (Lee et al., 1993; Yu and Yan, 2015; Thiery-Antier et al., 2016), which is called sepsis-related thrombocytopenia (SRT) with the incidence rate of 35%–59% and mortality rate of 13%–83% (Sharma et al., 2007; Levi and Löwenberg, 2008). However, SRT as a complication closely related to the prognosis of sepsis patients, the mechanism and treatment of which are still controversial.

Many factors may contribute to the pathogenesis of SRT (Bedet et al., 2018). Endotoxemia and cytokines in patients with sepsis may activate platelets (PLT) (Marshall, 2010; Schrottmaier et al., 2016), and increase the interaction of platelets with leukocytes, including platelet adhesion (Seeley et al., 2012). Thrombopoietin (TPO) and interleukin (IL)-6 significantly increased in septic patients which promoted the activation of platelet (Shimizu et al., 2018). Platelet counts (PCs) may be reduced observably due to platelet consumption and activation. Besides, thrombocytopenia may be due to the migration of platelets to the lungs, liver, and bone marrow during sepsis (Vincent et al., 2002; Koyama et al., 2018). And the decreased production of platelets and immune-mediated thrombocytopenia may also contribute to the SRT (Larkin et al., 2016). However, the complex mechanism limited the treatment of SRT.

The treatment of SRT involves treating the infection, platelet transfusion, intravenous immunoglobulin (IVIG), and administration of platelet-elevating drugs (Kuter and Begley, 2002; Naime et al., 2018; Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association, 2020). Due to the shortage of resources and the risk of blood transfusion, the clinical application of platelet transfusion was limited (Heyman and Schiffer, 1990; Nieken et al., 1995). As we knew, granulocyte macrophage colony-stimulating factor (GM-CSF), recombinant human IL-6 (rhIL-6), and recombinant human IL-11 (rHuIL-11) were used to promote platelet production. However, due to mild thrombopoiesis activity and clinically unacceptable adverse effects, the use of which were also limited (Nieken et al., 1995). And currently IVIG is not recommended for the treatment of SRT (Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association, 2020).

Recombinant human thrombopoietin (rhTPO), similar to endogenous TPO, is a recombinant form of the c-MPL ligand, which has been shown to effectively increase PCs (Vadhan-Raj et al., 2005). And it is widely used in chemotherapy or immune-related thrombocytopenia, with curative effects and less adverse effects (Wang et al., 2012; Kuter, 2015). Wu Q et al. reported that rhTPO would increase the PCs in SRT patients and reduce the platelet transfusion effectively (Wu et al., 2014). However, studies also suggested that blocking TPO may be helpful in reducing organ damage in sepsis patients (Cuccurullo et al., 2016; Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association, 2020). Thus, it was still inconclusive whether rhTPO can improve the prognosis of sepsis patients with thrombocytopenia. The objective of this study was to clarify the efficacy and safety of rhTPO on SRT by pooled the published randomized controlled trials (RCTs).

MATERIALS AND METHODS

The present systematic review and meta-analysis were reported in accordance with the Preferred Reporting item for Systematic Review and Meta-analysis (PRISMA) statement (Liberati et al., 2009).

Search Strategy

PubMed, Cochrane Library, Embase, China National Knowledge Infrastructure (CNKI), and Wanfang Database were electronically searched to RCTs about rhTPO for treating sepsis patients with thrombocytopenia from inception to March 4, 2020, regardless of language and region. We used the combination of keywords and terms to retrieve each database. In addition, the reference lists of related literature were manually searched for possible trials. The search strategy for PubMed is shown in **Supplementary Table 1**.

Selection Criteria

Two authors (JZ and ZL) searched independently, according to predefined inclusion and exclusion criteria. First, duplicate literature deletion, title, and abstract screening for relevance were been done using Endnote software. Then, the full-text was acquired to determine inclusion eligibility. Any disagreement would be resolved through discussion, a third review author (MY) would participate in where necessary.

Published literature were included by meeting the following criteria: 1. population: Adult patients with sepsis, severe sepsis, or septic shock, and combining with thrombocytopenia (PLT < 100×10^9/L) (Shankar-Hari et al., 2016). 2. intervention: recombinant human thrombopoietin. 3. comparison: conventional antibiotic therapy, or the former combined with IVIG. 4. design: randomized controlled trials.

Outcomes and Data Extraction

Two authors independently extracted data using a pre-piloted form designed by Excel 2019 software (Microsoft Corporation) and the result confirmed by another author. The collected data include: the first author, publish year, study period, sample size, mean age and sex ratio of each group, and the level of PLT at admission, Acute Physiology, Age, Chronic Health Evaluation II (APACHE II) scores at admission, and outcomes data. If any information above is inadequate, we contacted the original author *via* email to consult related data. We resolved discrepancies through discussion. The predefined primary outcomes were the level of PLT on the 7^{th} day of treatment. The secondary outcomes were the 28-d mortality, the length of activated partial thromboplastin time (APTT) and prothrombin time (PT) on 7^{th} day, the levels of glutamic-pyruvic transaminase (ALT) and creatinine (Cr) on the 7^{th} day, the total transfusion amounts of red blood cells, plasma, and platelet during hospitalization, and the length of intensive care unit (ICU) stay.

Quality Assessment

The quality of filtered articles was been assessed by two authors respectively. Cochrane Handbook for Systematic Reviews of Interventions (5.1.0) was used to assess the risk of bias for RCTs, which contain seven aspects: random sequence generation, allocation concealment, performance bias, detection bias, attrition bias, reporting bias, and other bias (Higgins et al., 2011). We reviewed each RCT and divided them into the high, low, or unclear risk of bias. Trial with more than one high-risk aspect was considered as a high risk of bias whereas trial with low risk of bias for all aspects was considered to be at low risk of bias, otherwise, it was considered as an unclear risk of bias.

Quality of Evidence

Two authors assessed the quality of each evidence respectively by using the GRADE system (Grading of Recommendations Assessment, Development, and Evaluation) for risk of bias, inconsistency, indirectness, imprecision, and publication bias (Guyatt et al., 2008). The quality was divided into very low, low, moderate, or high. The results were generated by using the GRADE Profiler.

Statistical Analysis

For dichotomous data, we calculated the relative risks (RRs) with 95% confidence intervals (CIs) by using Mantel-Haenszel method, regardless of the type of effect models. For continuous data, we calculated the standard mean difference (SMD) and 95% CIs. P values less than 0.05 were considered to be significant. Heterogeneity across trials was examined by using the I^2 statistical tests as well as P values. Those with $P < 0.1$ and I^2 greater than 50% seemed as significant heterogeneity, we used a random-effect model to get an overall summary. However, the fixed-effect model would be performed when the result of the heterogeneity test show that $P \geqq 0.1$ or $I^2 \leqq 50\%$. The sensitivity analysis was carried out by the leave-one-out method to explore the sources of heterogeneity and tested the stability of results. Publication bias was detected by the funnel plot qualitatively and also quantitatively assessed by using the test of Egger's. All statistical analyses were performed using Revman software (version 5.3).

RESULTS

Literature Research

The flow diagram shows the process of literature screening, selection, and reasons for exclusion (**Figure 1**). Our initial search yielded 166 records. After removing duplications and reviewing the titles/abstracts by using Endnote, 28 articles were thought to be potentially eligible for inclusion. After reading the full-text, 18 studies were excluded for the following reasons: study protocol (n=2); population doesn't meet the criterion (n=4); cohort study (n=6); review (n=1); conference paper (n=1); intervention measures inconsistent(n=2); duplication of records (n=1); only one author (n=1). As a result, 10 studies (Gao et al., 2011; Li et al., 2013; Li, 2015; Yang et al., 2015; Qi et al., 2016; Zhang et al., 2016; Feng et al., 2018; Zhang et al., 2018; Wang et al., 2019; Yan et al., 2019) were eventually included in this meta-analysis.

Trials Characteristics

Characteristics of included trials were summarized in **Table 1**. The 10 included trials were published from 2011 to 2019, with the sample sizes range from 43 to 102, with a total of 681 participates. For the treatment in control group, 6 trials adopted conventional antibiotic therapy (Li et al., 2013; Yang et al., 2015; Qi et al., 2016; Zhang et al., 2016; Zhang et al., 2018; Yan et al., 2019), 4 trials used the conventional antibiotic therapy combined with IVIG in addition (Gao et al., 2011; Fu and Zhang, 2017; Feng et al., 2018; Wang et al., 2019). The dosage of rhTPO was 300 U/kg/d in most trials, however, there are 2 trials performed 15,000 U/d (Qi et al., 2016; Zhang et al., 2018). There was no statistical difference between baseline data between rhTPO and control groups in each trial.

Risk of Bias Assessment and GRADE Profile Evidence

Supplementary Table 2 shown the details of each risk of bias. On the whole, though, no one in these included trials, had detailed whether blinding for participates, personnel, and outcome assessment was performed, and just two trials reported the allocation concealment. Thus, we had to classify all trials included as unclear risk of bias, according to Cochrane Handbook.

GRADE evidence profiles are shown in **Supplementary Figures 1** and **2**. Overall, the primary outcome was categorized as low-quality evidence. Except for the transfusion of blood products (rhTPO vs conventional antibiotic therapy) were graded as very low-quality evidence due to high heterogeneity, the other secondary outcomes were considered as low-quality.

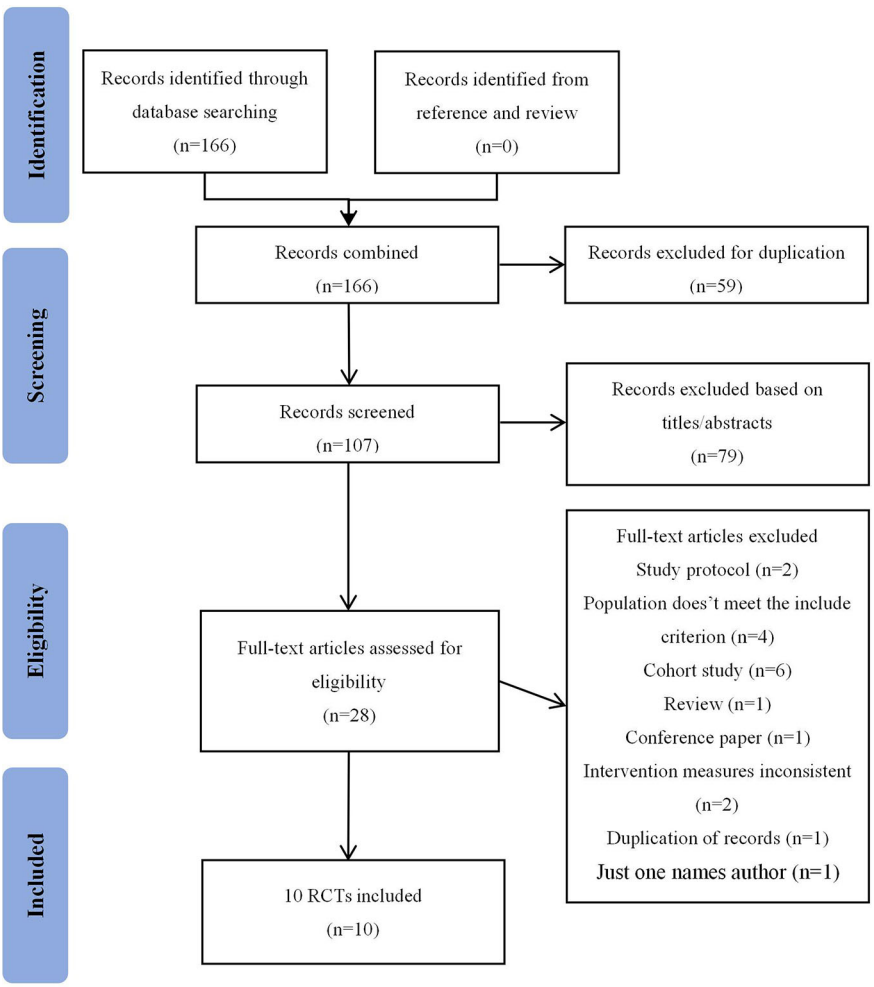

FIGURE 1 | Flow diagram of study selection.

rhTPO vs Conventional Antibiotic Therapy

Primary Outcomes

Six studies reported the information on the level of PLT on 7th day of treatment with totaling 394 patients (Li et al., 2013; Yang et al., 2015; Qi et al., 2016; Zhang et al., 2016; Zhang et al., 2018; Yan et al., 2019). Compared with conventional antibiotic therapy, rhTPO significantly increased the PCs on 7th after treatment (SMD, 2.61; 95%CI, 1.28–3.94; P < 0.001) with high heterogeneity (I^2 = 96%). Then, we performed subgroup analysis according to different dosages, which found that the results didn't influence by dosage (**Figure 2A**). However, there was a possible publication bias detected by Egger's test (P=0.007), the funnel plot was shown in **Figure 3**.

Secondary Outcomes

The 28-d Mortality

Four trials reported the information about 28-d mortality with totaling 250 patients (Li et al., 2013; Qi et al., 2016; Zhang et al., 2016; Zhang et al., 2018). Compared with conventional antibiotic therapy, rhTPO significantly decreased the 28-d mortality (RR, 0.66; 95%CI, 0.46–0.97; P=0.03) with a low heterogeneity (I^2 = 40%) (**Supplementary Figure 3**).

Coagulation Indicators

Three trials reported the information about the length of APTT (Li et al., 2013; Qi et al., 2016; Zhang et al., 2018) and four trials reported the length of PT (Li et al., 2013; Yang et al., 2015; Qi et al., 2016; Zhang et al., 2018) on the 7th day after treatment. However, there was no significant difference in the length of APTT (SMD, −0.12; 95%CI, −0.41–0.17; P=0.43) and PT (SMD, −0.21; 95%CI, −0.47–0.04; P=0.1) on the 7th day after treatment, when compared rhTPO with conventional antibiotic therapy. And the heterogeneity of these results was very low (I^2 = 0%) (**Supplementary Figure 4**).

Hepatic and Renal Function Indicators

Five trials reported the level of ALT (Li et al., 2013; Yang et al., 2015; Qi et al., 2016; Zhang et al., 2016; Zhang et al., 2018) and

TABLE 1 | The characteristics of included randomized control trials.

First author (published year)	Study period	Sample size		Mean age (Year)		Interventions		The level of PLT at admission ($x10^9$/L)		APACHE II at admission		Outcomes
		rhTPO group	Control group	rhTPO group	Control group	rhTPO group	Control group	rhTPO group	Control group	rhTPO group	Control group	
Feng et al. (2018)	2011.09-2013.09	63	39	57.2 ± 21.2	56.9 ± 18.3	300 U/kg/d	CAT+IVIG	28.7 ±9.7	27.5 ± 14.1	22.6 ± 6.1	23.0 ± 4.6	①②③④⑨
Gao et al. (2011)	2009.01-2009.11	21	22	43.10 ± 21.25	41.74 ± 17.65	300 U/kg/d	CAT+IVIG	25.14 ± 7.09	26.13 ± 7.11	21.93 ± 8.34	23.47 ± 10.26	①②③④⑩
Li (2015)	2012.01-2014.03	32	35	58.56 ± 25.43	59.09 ± 23.89	300 U/kg/d	CAT+IVIG	36.93 ± 5.50	35.26 ± 4.71	26.94 ± 5.74	24.03 ± 6.35	①②③④⑨
Li et al. (2013)	2010.01-2011.12	28	20	NA	NA	300 U/kg/d	CAT only	34.78 ± 4.77	36.60 ± 4.25	NA	NA	①②③④⑤
Qi et al. (2016)	2015.01-2015.10	30	30	50.3 ± 26.2	50.9 ± 25.7	15,000 U/d	CAT only	52.83 ± 16.32	52.11 ± 16.29	18.8 ± 2.7	18.1 ± 2.2	⑥⑦⑧⑨⑩
Yan et al. (2019)	2016.01-2017.12	42	42	59.13 ± 0.37	59.14 ± 0.39	300 U/kg/d	CAT only	25.49 ± 2.53	25.52 ± 2.51	18.35 ± 2.14	18.31 ± 2.16	①②③④⑤
Yang et al. (2015)	2014.01-2014.12	30	30	NA	NA	300 U/kg/d	CAT only	34.98 ± 0.64	34.31 ± 0.78	NA	NA	①②③④⑤
Zhang et al. (2018)	2016-2018	34	42	54.50 ± 19.53	53.65 ± 15.52	15,000 U/d	CAT only	30.64 ± 10.19	37.17 ± 1.68	20.21 ± 7.10	19.78 ± 6.05	①②③④⑤
Zhang et al. (2016)	2013.10-2015.09	35	31	56 ± 9	54 ± 8	300 U/kg/d	CAT only	37 ± 8	38 ± 19	17 ± 3	17 ± 3	⑥⑦⑧⑨⑩
Wang et al. (2019)	2011.09-2013.09	63	39	57.2 ± 21.2	56.9 ± 18.3	300 U/kg/d	CAT+IVIG	28.7 ± 9.7	27.5 ± 14.1	22.6 ± 6.1	23.0 ± 4.6	①②③④⑩

rhTPO, recombinant human thrombopoietin; CAT, conventional antibiotic therapy; IVIG, intravenous immunoglobulin; PLT, platelet; APACHE II, Acute Physiology, Age, Chronic Health Evaluation; NA, not available

Outcomes: ① The level of PLT on d7;② 28-d mortality;③ The level of APTT on d7;④ The level of PT on d7;⑤ The level of ALT on d7;⑥ The level of Cr on d7;⑦ The transfusion volume of red blood cells;⑧ The transfusion volume of plasma;⑨ The transfusion volume of platelet;⑩ Length of ICU stay.

four trials reported the level of Cr (Li et al., 2013; Yang et al., 2015; Qi et al., 2016; Zhang et al., 2018) on 7th day of treatment. The results of rhTPO group shown margin effectiveness in the term of ALT reduction (SMD, −0.22; 95%CI, −0.45–0, P=0.05), when compared with conventional antibiotic therapy. However, there was no statistical difference in the level of Cr (SMD, 0.04; 95%CI, −0.21–0.30; P=0.74) between the two groups. The heterogeneity of both outcomes was low (I^2 = 31% and 0.0% respectively) (**Supplementary Figure 5**).

Transfusion of Blood Products

There were five studies reported the transfusion volume of platelet (Li et al., 2013; Yang et al., 2015; Qi et al., 2016; Zhang et al., 2016; Zhang et al., 2018), four studies reported the transfusion volume of red blood cells and plasma (Li et al., 2013; Qi et al., 2016; Zhang et al., 2018; Yan et al., 2019). Comparing with conventional antibiotic therapy, rhTPO significantly decreased the transfusion volume of platelet (SMD, −1.47; 95%CI, −1.99–−0.96; P < 0.001), red blood cells (SMD, −1.42; 95%CI, −2.51–−0.34; P=0.01) and plasma (SMD, −2.35; 95%CI, −4.14–−0.56; P=0.01), and an obvious high heterogeneity was observed in the results (I^2 = 77%, 93%, 97% respectively) (**Figure 4**).

Length of ICU Stay

Four trials reported the length of ICU stay (Li et al., 2013; Qi et al., 2016; Zhang et al., 2016; Zhang et al., 2018) with totaling 250 patients. The pooled result has shown that rhTPO significantly reduce the length of ICU stay (SMD, −0.31; 95% CI, −0.56–−0.0; P=0.02) compared with conventional antibiotic therapy, with a low heterogeneity (I^2 = 0%) (**Supplementary Figure 6**).

rhTPO vs Conventional Antibiotic Therapy + IVIG

Primary Outcome

Three trials reported the information about the level of PLT on the 7th day of treatment in both rhTPO group and conventional antibiotic therapy combine with IVIG group (Gao et al., 2011; Li, 2015; Feng et al., 2018). The result of meta-analysis shown that rhTPO could increase the PCs on the 7th day of treatment when compared with the control group, and the difference was statistically significant (SMD, 0.86; 95%CI, 0.54–1.17; P < 0.001) (**Figure 2B**), the heterogeneity was low (I^2 = 22%). No publication bias has been found with Egger's test (P=0.684) and funnel plot (**Figure 3**).

Secondary Outcomes

We just conducted pooled analysis for the 28-d mortality (Gao et al., 2011; Li, 2015; Wang et al., 2019), blood products transfusion (Gao et al., 2011; Li, 2015; Feng et al., 2018; Wang et al., 2019), and the length of ICU stay (Gao et al., 2011; Wang et al., 2019), due to the limited relate data. Comparing with conventional antibiotic therapy combine with IVIG group, the meta-analysis shown that rhTPO could significantly decreased the transfusion of volume of platelet

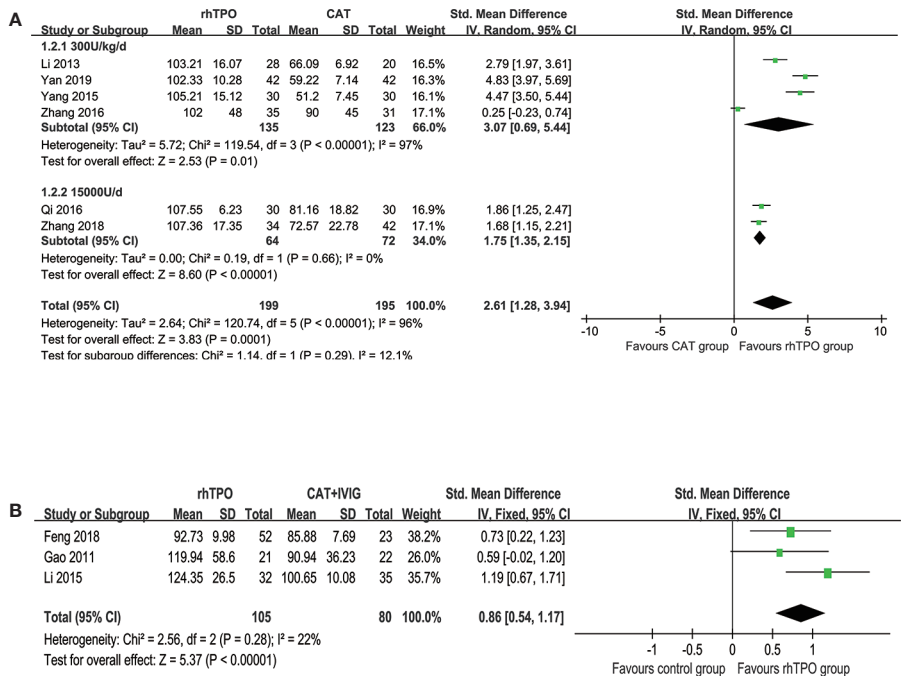

FIGURE 2 | The forest plot for the level of platelet on the 7th day of treatment. **(A)** rhTPO vs conventional antibiotic therapy. **(B)** rhTPO vs conventional antibiotic therapy+ IVIG.

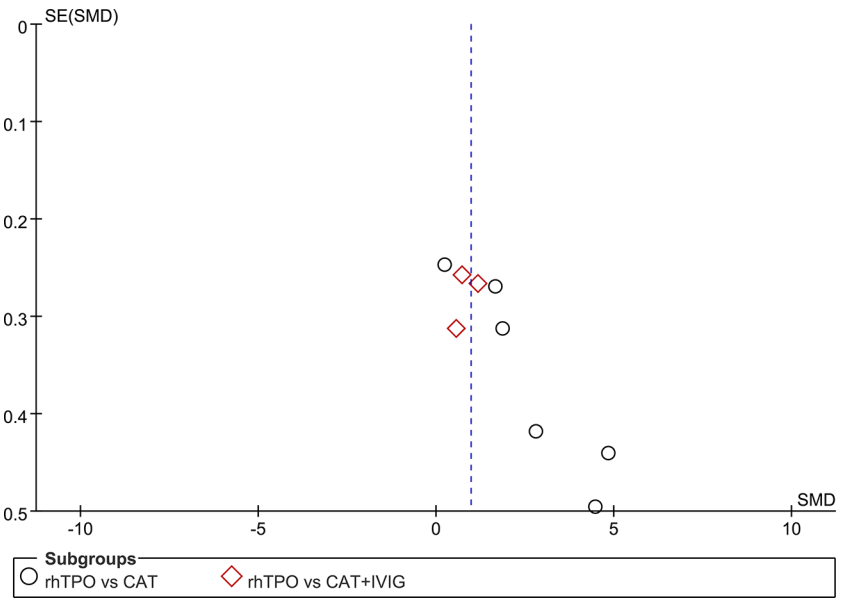

FIGURE 3 | Test for publication bias for the primary outcome.

(SMD, −0.65; 95%CI, −0.89–−0.40; P < 0.001), red blood cells (SMD, −0.47; 95%CI, −0.72–−0.23; P < 0.001) and plasma (SMD, −0.61; 95%CI, −0.85–−0.36; P < 0.001), with the low heterogeneity (I^2 = 0.0%, 49% and 34% respectively). However, the two arms didn't differ with the respect to the 28-d mortality (RR, 0.82; 95%CI, 0.54–1.24; P=0.34) and the length of ICU stay (SMD, −0.02; 95%CI, −0.35–0.31; P=0.90). The results are shown in **Table 2**.

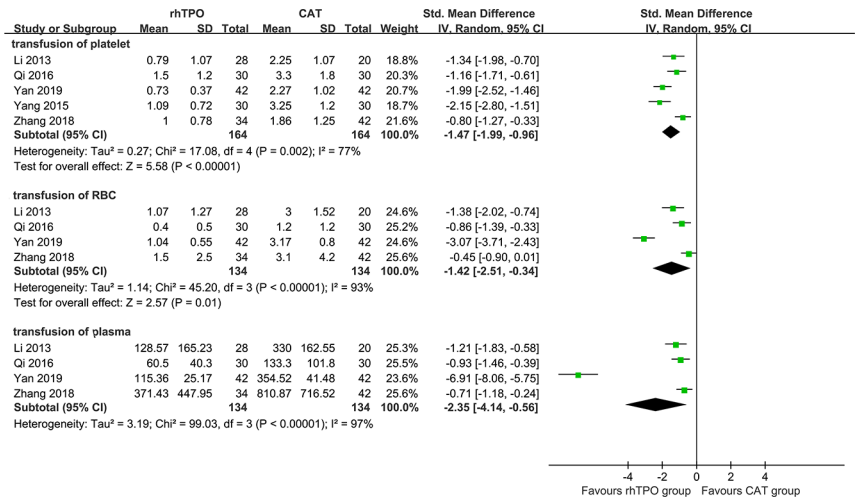

FIGURE 4 | The forest plot for the transfusion of blood products (rhTPO vs conventional antibiotic therapy).

TABLE 2 | The pooled results of secondary outcomes (rhTPO vs conventional antibiotic therapy+IVIG).

Outcomes	Included trials	Heterogeneity		Effects model	Pooled results		
		P values	I^2		RR/SMD values	95%CIs	*P* values
28-d morality	3 (Gao et al., 2011; Li, 2015; Wang et al., 2019)	0.7	0.0%	Fixed-effects model	RR=0.82	0.54, 1.24	0.34
Transfusion of platelet	4 (Gao et al., 2011; Li, 2015; Feng et al., 2018; Wang et al., 2019)	0.9	0.0%	Fixed-effects model	SMD=−0.65	−0.89, −0.40	<0.001
Transfusion of RBC	4 (Gao et al., 2011; Li, 2015; Feng et al., 2018; Wang et al., 2019)	0.12	49%	Fixed-effects model	SMD=−0.47	−0.72, −0.23	<0.001
Transfusion of plasma	4 (Gao et al., 2011; Li, 2015; Feng et al., 2018; Wang et al., 2019)	0.21	34%	Fixed-effects model	SMD=−0.61	−0.85, −0.36	<0.001
Length of ICU stay	2 (Gao et al., 2011; Wang et al., 2019)	0.23	31%	Fixed-effects model	SMD=−0.02	−0.35, 0.31	0.90

Sensitively Analysis

We found that high heterogeneity appeared when rhTPO compared with conventional antibiotic therapy. For the level of PLT on the 7th day of treatment, the I^2 value decreased to 88% after Yan et al. (2019) and Zhang et al. (2016) excluded, however, the pooled result was stable by using sensitively analysis. For the transfusion of platelet, we found that the I^2 value decreased to 62.5% when Zhang et al. (2018) excluded. For the transfusion of red blood cells, the I^2 value decreased to 64% after Yan et al. (2019) excluded, while the pooled result was changed after Li et al. (2013) removed during sensitive analysis. For the transfusion of plasma, we found that the I^2 value decreased to 0% after Yan et al. (2019) excluded. Thus, we believe that the high heterogeneity may arise from the following factors: sample size, the quality of the included trial, and the difference in dosage of rhTPO.

DISCUSSION

In this systematic review and meta-analysis of RCTs, compared with conventional antibiotic therapy alone, conventional antibiotic therapy plus rhTPO could significantly increase PCs, and reduce 28-d mortality, transfusion volume of blood products, and the length of ICU stay. And also proved that PCs was improved on the 7th of treatment, reduced transfusion volume of blood products and didn't increased adverse events when compared with conventional antibiotic therapy combined with IVIG.

The study demonstrated that early control of triggering thrombocytopenia was the prerequisite for treatment (Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association, 2020). Even rhTPO would also be a potential therapeutic drug for SRT based on current evidence, effective infection control was the cornerstone of SRT treatment (Larkin et al., 2016). ICU patients with thrombocytopenia are at a high risk of bleeding, receiving transfusions, and death (Williamson et al., 2013). An acute or sustained reduction in PCs always suggests a poor prognosis (Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association, 2020). Akca et al. reported that the PCs had been decreased for 14 d, the mortality rate of this disease would be 66% in critically ill patients (Akca et al., 2002). Nijsten et al. also suggested that

slow rise of PCs in ICU patients would indicate a worse outcome (Nijsten et al., 2000). In this study, the PCs was significantly improved on the 7th of rhTPO treatment, which may be related to the time required for TPO to promote the proliferation and division of megakaryocytes into PLT (Kaushansky, 2009). Rapid improvement of PCs and shorting of the time to reach the target PLT all would be helpful in reducing bleeding, blood transfusion, and mortality (Akca et al., 2002). Patients with thrombocytopenia always need prolonged vasopressor support and ICU stay (Venkata et al., 2013). The administration of rhTPO could successfully reduce the length of ICU stay and lower the total hospitalization cost due to the effective improvement of SRT (Wang et al., 2019).

Many causes may contribute to the development of thrombocytopenia in ICU. There might be several reasons for rhTPO to increase PCs in SRT patients. First, the production of platelets mainly depends on the maturation and proliferation of bone marrow megakaryocytes, and was influenced by TPO concurrently (Fu and Zhang, 2017). rhTPO can stimulate the formation and differentiation of bone marrow megakaryocytes, and promote the formation of megakaryocytes in all stages, then produces active platelets (Zhang et al., 2016). Studies suggested that rhTPO would promote the proliferation and division of bone marrow megakaryocytes into mature platelets in sepsis, and increased PCs in peripheral blood (Jiang et al., 2019). Second, sepsis involved inflammation initiation and amplification, endothelial dysfunction, platelet activation and aggregation, and coagulation imbalance, which was characterized by the interaction between endothelial cells and activated platelets (Wang et al., 2011; Vardon Bounes et al., 2018). Activated platelets played a key role in the development of sepsis by participating in the activation of inflammation and coagulation pathways (Vandijck et al., 2010). rhTPO might inhibit platelet activation in SRT, weakened the interaction between endothelial cells and activated platelets, and increased PCs (Cloutier et al., 2018). Moreover, PCs may be reduced due to both the platelets' migration to lungs and liver and bone marrow during sepsis (Vincent et al., 2002; Koyama et al., 2018). Studies have shown that about 14% platelets were sequestrated in the lung tissue in sepsis (Cloutier et al., 2018). The administration of rhTPO could reduce platelet sequestration in sepsis and increase PCs (Jiang et al., 2019). Our results support the application value of rhTPO in SRT patients, and its mechanisms and standardized treatment needs to be further investigated.

IVIG is the main therapeutic drug for immune-related thrombocytopenia (Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association, 2020). It is thought to modulate the immune responses associated with sepsis by binding and neutralizing circulating toxins, and also used in SRT (AL-Rawi et al., 2009). However, due the risk of infectious diseases transmission and the high cost of IVIG, the use is limited (Wang et al., 2006). Currently IVIG is not recommended for the treatment of SRT (Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association, 2020). In this study, we found that rhTPO was better than IVIG in improving PCs on the 7th of treatment and reducing transfusion volume of blood products, and didn't increased adverse events. And the cost of the rhTPO treatment is lower than IVIG obviously.

Fever, rash, dizziness, pain at the injection site, and elevated blood pressure were the most common adverse reactions of administration of rhTPO reported in prior studies (Zhou et al., 2015). Furthermore, thrombosis was the main risk of using thrombopoiesis agents (Mahévas et al., 2016). In the all 10 RCTs included, there was no adverse reaction and thromboembolic events reported, which suggested that rhTPO was a safe treatment for SRT.

LIMITATIONS

There were several limitations to this study. First, no blinding was taken place in the studies. But considering the outcome indicators are objective, it may be no impact on results. Second, we found that high heterogeneity appeared when rhTPO compared with conventional antibiotic therapy, and the high heterogeneity may arise from the sample size, the quality of the included trial, and the difference in dosage of rhTPO. Third, the quality of the included literature was low, the sample size was small, and the control was not uniform. In the future, more well-designed RCTs are needed to verify the safety and efficacy of rhTPO on SRT. And the timing of intervention, the course of treatment, the long-term efficacy, and safety need further study. At present, a randomized, multi-center, controlled trial named RESCUE (NCT02707497) is being conducted in Shanghai, which is aims to further investigate that whether the administration of rhTPO is effective and safe therapy on acute severe SRT (Zhou et al., 2019).

CONCLUSIONS

Current evidence has shown rhTPO would increase PCs on the 7th day of treatment and reduced the transfusion volume of blood products in SRT during hospitalization. There was no adverse reaction and thromboembolic events reported in all included studies. The conclusions are needed to be verified indeed by more multicenter RCTs due to the limitation of the included studies.

AUTHOR CONTRIBUTIONS

This study was designed by JZ and MY. ZL, JZ, and MY contributed to the literature searching, abstracts reading, data extracting and statistical analyses. The first draft of the essay was written by JZ and ZL. WX and YZ offered some practical suggestions and contributed to the writing of the essay. TH and MY revised the article critically.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00940/full#supplementary-material

REFERENCES

Akca, S., Haji-Michael, P., de Mendonca, A., Suter, P., Levi, M., and Vincent, J-L. (2002). Time course of platelet counts in critically ill patients. *Crit. Care Med.* 30 (4), 753–756. doi: 10.1097/00003246-200204000-00005

AL-Rawi, S., Woodward, L. J., and Knight, J. (2009). Puerperal streptococcal toxic shock syndrome treated with recombinant human activated protein C and intravenous immunoglobulin. *Inter. J. Obstent Anesth.* 18 (2), 169–172. doi: 10.1016/j.ijoa.2008.10.003

Bedet, A., Razazi, K., Boissier, F., Surenaud, M., Hue, S., Giraudier, S., et al. (2018). Mechanisms of thrombocytopenia during septic shock: a multipex cluster analysis of endogenous sepsis mediators. *Shock* 49 (6), 641–648. doi: 10.1097/SHK.0000000000001015

Cloutier, N., Allaeys, I., Marcoux, G., Marcoux, G., Machlus, K. R., Mailhot, B., et al. (2018). Platelets release pathogenic serotonin and return to circulation after immune complex-mediated sequestration. *Proc. Natl. Acad. Sci. U.S.A.* 115 (7), E1550–E1559. doi: 10.1073/pnas.1720553115

Critical Care Medicine Committee of Chinese PLA and Chinese Society of Laboratory Medicine, Chinese Medical Association (2020). Expert consensus for diagnosis and treatment of thrombocytopenia in adult critical illness in China. *Med. J. Chin. PLA* 45 (5), 457–474. doi: 10.11855/j.issn.0577-7402.2020.05.01

Cuccurullo, A., Greco, E., Lupia, E., De Giuli, P., Bosco, O., Martin-Conte, E., et al. (2016). Blockade of thrombopoietin reduces organ damage in experimental endoxemia and polymicrobial sepsis. *PloS One* 11 (3), e0151088. doi: 10.1371/journal.pone.0151088

Feng, S. Q., Gu, J. P., and Yu, Y. (2018). Efficacy and safety of recombinant human thrombopoietin in treatment of sepsis-associated thrombocytopenia. *China Rural Health* 14, 10–11. doi: 10.3969/j.issn.1674-361x.2018.06.019

Fleischmann, C., Scherag, A., Adhikari, N. K., Hartog, C. S., Tsaganos, T., Schlattmann, P., et al. (2016). Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am. J. Respir. Crit. Care Med.* 193 (3), 259–272. doi: 10.1164/rccm.201504-0781OC

Fu, H. P., and Zhang, Y. X. (2017). Mechanism of platelet dysfunction in patients with sepsis. *China J. Crit. Care Med.* 37 (10), 873–877. doi: 10.3969/j.issn.1002-1949.2017.10.001

Gao, H. M., Wang, B., Li, Y., and Wang, Y. Q. (2011). The clinical study of rhTPO used for treatment of sepsis-associated thrombocytopenia. *Chin. J. Emerg. Med.* 20 (3), 239–243. doi: 10.3760/cma.j.issn.1671-0282.2011.03.005

Guyatt, G. H., Oxman, A. D., Vist, G. E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P., et al. (2008). GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 336 (7650), 924–926. doi: 10.1136/bmj.39489.470347.AD

Heyman, M. R., and Schiffer, C. A. (1990). Platelet transfusion therapy for the cancer patient. *Semin. Oncol.* 17 (2), 198–209. doi: 10.1016/0167-8140(90)90010-T

Higgins, J. P., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343, d5928. doi: 10.1136/bmj.d5928

Jiang, J., Wang, B., Yu, X., Xu, H., and Wang, Y. (2019). Effect of recombinant human thrombopoietin on endotoxin-induced thrombocytopenia in mice. *Chin. Crit. Care Med.* 31 (9), 1108–1112. doi: 10.3760/cma.j.issn.2095-4352.2019.09.010

Kaushansky, K. (2009). Determinants of platelet number and regulation of thrombopoiesis. *Hematol. Am. Soc. Hematol. Educ. Program.* 1 (1), 147–152. doi: 10.1182/asheducation-2009.1.147

Koyama, K., Katayama, S., Muronoi, T., Tonai, K., Goto, Y., Koinuma, T., et al. (2018). Time course of immature platelet count and its relation to thrombocytopenia and mortality in patients with sepsis. *PloS One* 13 (1), e0192064. doi: 10.1371/journal.pone.0192064

Kuter, D. J., and Begley, C. G. (2002). Recombinant human thrombopoietin: basic biology and evaluation of clinical studies. *Blood* 100 (10), 3457–3469. doi: 10.1182/blood.V100.10.3457

Kuter, D. J. (2015). Managing thrombocytopenia associated with cancer chemotherapy. *Oncol. (Williston Park)* 29 (4), 282–294.

Larkin, C. M., Santos-Martinez, M. J., Ryan, T., and Radomski, M. W. (2016). Sepsis-associated thrombocytopenia. *Thromb. Res.* 141, 11–16. doi: 10.1016/j.thromres.2016.02.022

Lee, K. H., Hui, K. P., and Tan, W. C. (1993). Thrombocytopenia in sepsis: a predictor of mortality in the intensive care unit. *Singapore Med. J.* 34 (3), 245–246. doi: 10.1097/00003246-199906000-00029

Levi, M., and Löwenberg, E. C. (2008). Thrombocytopenia in critically ill patients. *Semin. Thromb. Hemost.* 34 (5), 417–424. doi: 10.1055/s-0028-1092871

Li, Y., Wang, Y. Q., and Wang, B. (2013). Effect of recombinant human thrombopoietin and interleukin-11 in the treatment of septic-related thrombocytopenia. *J. Tianjin Med. Univ.* 19 (2), 114–117,120. doi: 10.3969/j.issn.1006-8147.2013.02.009

Li, Y. (2015). Clinical study of recombinant human thrombopoietin and immunoglobulin in treatment of sepsis associated thrombocytopenia. *Drugs Clinic.* 30 (3), 313–317. doi: 10.7501/j.issn.1674-5515.2015.03.019

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, G., Gøtzsche, P. C., Ioannidis, J. P. A., et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J. Clin. Epidemiol.* 62 (10), e1–34. doi: 10.1016/j.jclinepi.2009.06.006

Mahévas, M., Gerfaud-Valentin, M., Moulis, G., Terriou, L., Audia, S., Guenin, S., et al. (2016). Characteristics, outcome, and response to therapy of multirefractory chronic immune thrombocytopenia. *Blood* 128 (12), 1625–1630. doi: 10.1182/blood-2016-03-704734

Marshall, J. C. (2010). Endotoxin in the pathogenesis of sepsis. *Contrib Nephrol.* 167, 1–13. doi: 10.1159/000315914

Naime, A. C. A., Ganaes, J. O. F., and Lopes-Pires, M. E. (2018). Sepsis: the involvement of platelets and the current treatments. *Curr. Mol. Pharmacol.* 11 (4), 261–269. doi: 10.2174/1874467211666180619124531

Nieken, J., Mulder, N. H., Buter, J., Vellenga, E., Limburg, P. C., Piers, D. A., et al. (1995). Recombinant human interleukin-6 induces a rapid and reversible anemia in cancer patients. *Blood* 86 (3), 900–905. doi: 10.1182/blood.V86.3.900.900

Nijsten, M. W., ten Duis, H. J., Zijlstra, J. G., Prote, R. J., Zwaveling, J. H., Paling, J. C., et al. (2000). Blunted rise in platelet count in critically ill patients is associated with worse outcome. *Crit. Care Med.* 28 (12), 3843–3864. doi: 10.1097/00003246-200012000-00017

Qi, H., Meng, C. Q., Shang, Y., Yuan,S. Y., and Yao, S. L. (2016). The efficacy and safety of rhTPO in the treatment of sepsis-associated thrombocytopenia. *J. Clin. Emergency (China)* 17 (9), 663–667. doi: 10.1320/j.issn.1009-5918.2016.09.002

Schrottmaier, W. C., Kral, J. B., Zeitlinger, M., Salzmann, M., Jilma, B., and Assinger, A. (2016). Platelet activation at the onset of human endotoxemia is undetectable in vivo. *Platelets* 27 (5), 479–483. doi: 10.3109/09537104.2015.1119814

Seeley, E. J., Matthay, M. A., and Wolters, P. J. (2012). Inflection points in sepsis biology: from local defense to systemic organ injury. *Am. J. Physiol. Lung Cell Mol. Physiol.* 303 (5), L355–L363. doi: 10.1152/ajplung.00069.2012

Shankar-Hari, M., Phillips, G. S., Levy, M. L., Seymour, C. W., Liu, V. X., Deutschman, C. S., et al. (2016). Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 315 (8), 775–787. doi: 10.1001/jama.2016.0289

Sharma, B., Sharma, M., Majumder, M., Steier, W., Sangal, A., and Kalawar, M. (2007). Thrombocytopenia in septic shock patients- a prospective observational

study of incidence, risk factors and correlation with clinical outcome. *Anaesth. Intensive Care* 35 (6), 874–880. doi: 10.1177/0310057X0703500604

Shimizu, M., Konishi, A., and Nomura, S. (2018). Examination of biomarker expressions in sepsis-related DIC patients. *Int. J. Gen. Med.* 11, 353–361. doi: 10.2147/IJGM.S173684

Thiery-Antier, N., Binquet, C., Vinault, S., Meziani, F., Boisrame-Helms, J., and Quenot, J.-P. (2016). EPIdemiology of Septic Shock Group. Is thrombocytopenia an early prognostic marker in septic shock? *Crit. Care Med.* 44 (4), 764–772. doi: 10.1097/CCM.0000000000001520

Vadhan-Raj, S., Cohen, V., and Bueso-Ramos, C. (2005). Thrombopoietic growth factors and cytokines. *Curr. Hematol. Rep.* 4 (2), 137–144.

Vandijck, D. M., Blot, S. I., De Waele, J. J., Hoste, E. A., Vandewoude, K. H., and Decruyenaere, J. M. (2010). Thrombocytopenia and outcome in critically ill patients with bloodstream infection. *Heart Lung* 39 (1), 21–26. doi: 10.1016/j.hrtlng.2009.07.005

Vardon Bounes, F., Mémier, V., Marcaud, M., Jacquemin, A., Hamzeh-Cognasse, H., Garcia, C., et al. (2018). Platelet activation and prothrombotic properties in a mouse model of peritoneal sepsis. *Sci. Rep.* 8 (1), 13536. doi: 10.1038/s41598-018-31910-8

Venkata, C., Kashyap, R., Farmer, J. C., and Afessa, B. (2013). Thrombocytopenia in adult patients with sepsis: incidence, risk factors, and its association with clinical outcome. *J. Intensive Care* 1 (1), 9. doi: 10.1186/2052-0492-1-9

Vincent, J. L., Yagushi, A., and Pradier, O. (2002). Platelet function in sepsis. *Crit. Care Med.* 30 (5 Suppl), S313–S317. doi: 10.1097/00003246-200205001-00022

Wang, Y. Q., Dou, L., Zhang, H. Y., Zhao, B., Wang, D. H., and Cao, S. H. (2006). Treatment of septic thrombocytopenia with immunoglobulin. *Chin. J. Emerg. Med.* 15 (10), 905–908. doi: 10.3760/j.issn:1671-0282.2006.10.012

Wang, B., Wang, Y. Q., Cao, S. H., and Liang, Y. (2011). Platelet parameters and platelet Toll-like receptor 4 (TLR4) expression in patients with sepsis, and the effect of a joint treatment-plan integrating traditional Chinese and western medicine: a clinical study. *Chin. Crit. Care Med.* 23 (10), 616–620. doi: 10.3760/cma.j.issn.1003-0603.2011.10.014

Wang, S., Yang, R., Zou, P., Hou, M., Wu, D., Shen, Z., et al. (2012). A multicenter randomized controlled trial of recombinant human thrombopoietin treatment in patients with primary immune thrombocytopenia. *Int. J. Hematol.* 96 (2), 222–228. doi: 10.1007/s12185-012-1124-8

Wang, B., Yao, F., Wang, Y., Gao, H., Xing, Y., Fan, Z., et al. (2019). A multi-center clinical observation of recombinant human thrombopoietin for the treatment of sepsis-associated thrombocytopenia. *Int. J. Clin. Exp. Med.* 12 (5), 6324–6334.

Williamson, D. R., Albert, M., Heels-Ansdell, D., Arnold, D. M., Lauzier, F., Zarychanski, B., et al. (2013). Thrombocytopenia in critically ill patients receiving thromboprophylaxis: frequency, risk factors, and outcomes. *Chest* 144 (4), 1207–1215. doi: 10.1378/chest.13-0121

Wu, Q., Ren, J., Wu, X., Wang, G., Gu, G., Liu, S., et al. (2014). Recombinant human thrombopoietin improves platelet counts and reduces platelet transfusion possibility among patients with severe sepsis and thrombocytopenia: a prospective study. *J. Crit. Care* 29 (3), 362–366. doi: 10.1016/j.jcrc.2013.11.023

Yan, X. J., Zhang, W. K., and Hou, L. Y. (2019). Therapeutic effects of recombinant human thrombopoietin on sepsis-associated thrombocytopenia. *Chin. J. Pract. Medicine.* 46 (15), 91–93. doi: 10.3760/cma.j.issn.1674-4756.2019.15.030

Yang, H. W., Yu, Q. P., Ye, X. W., Li, L., and Liu, S. (2015). Effect Research of recombinant human thrombopoietin in patients with sepsis-associated thrombocytopenia. *Med. Innovation China* 12 (23), 1–3. doi: 10.3969/j.issn.1674-4985.2015.23.001

Yu, Y. H., and Yan, J. (2015). Effect of recombinant human thrombopoietin in the treatment of septic-related thrombocytopenia. *China J. Mod. Appl. Pharm.* 32 (8), 1028–1032. doi: 10.13748/j.cnki.issn1007-7693.2015.08.032

Zhang, W. F., Fang, J. J., Wang, G. B., Ni, Y. L., Li, H., Gong, S. J., et al. (2016). Clinical study of recombinant human thrombopoietin in patients with sepsis-associated thrombocytopenia. *Chin. J. Crit. Care Med. (Electronic Edition)* 9 (5), 300–308. doi: 10.3877/cma.j.issn.1674-6880.2016.05.004

Zhang, X. Y., Qi, W. J., Zhou, W. J., Liu, Q. F., Wang, X. H., and Yang, X. J. (2018). Clinical study of recombinant human thrombopoietin in patients with sepsis-associated thrombocytopenia. *Ningxia Med. J.* 40 (9), 784–787. doi: 10.13621/j.1001-5949.2018.09.0784

Zhou, H., Xu, M., Qin, P., Zhang, H., Yuan, C., Zhao, H., et al. (2015). A multicenter randomized open-label study of rituximab plus rhTPO vs rituximab in corticosteroid-resistant or relapsed ITP. *Blood* 125 (10), 1541–1547. doi: 10.1182/blood-2014-06-581868

Zhou, Z., Feng, T., Xie, Y., Huang, P., Xie, H., Tian, R., et al. (2019). The effect of recombinant human thrombopoietin (rhTPO) on sepsis patients with acute severe thrombocytopenia: a study protocol for a multicenter randomized controlled trial (RESCUE trial). *BMC Infect. Dis.* 19 (1), 780. doi: 10.1186/s12879-019-4388-2

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Registered Interventional Clinical Trials for Old Populations With Infectious Diseases on ClinicalTrials.gov: A Cross-Sectional Study

Lingmin Chen[1], *Menghua Wang*[2], *Yi Yang*[3], *Jing Shen*[4] *and Yonggang Zhang*[5,6]*

[1] Department of Anesthesiology and National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University & The Research Units of West China (2018RU012, Chinese Academy of Medical Sciences), Chengdu, China, [2] Department of Urology, Institute of Urology (Laboratory of Reconstructive Urology), West China Hospital, Sichuan University, Chengdu, China, [3] Department of Clinical Medicine, Gansu University of Traditional Chinese Medicine, Lanzhou, China, [4] Department of General Practice, International Hospital of Sichuan Province, West China Hospital of Sichuan University, Chengdu, China, [5] Department of Periodical Press and National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, Chengdu, China, [6] Chinese Evidence-based Medicine Center, West China Hospital, Sichuan University, Chengdu, China

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***Correspondence:**
Yonggang Zhang
jebm_zhang@yahoo.com

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Background: Interventional clinical trials for infectious diseases in old population have arisen much attention in recent years, however, little is known about the characteristics of registered clinical trials regarding this field. This study aimed to investigate the characteristics of registered interventional trials for infectious diseases in old populations on ClinicalTrials.gov.

Methods: A cross-sectional study was performed. We used viral OR bacterial OR fungal OR parasitic OR infectious disease to search the ClinicalTrials.gov database and to assess characteristics of included trials. The age of participants was restricted to more than 65 years old. All analyses were performed using the SPSS19.0 software.

Results: A total of 138 registered trials were included. Among them, 105(76.1%) trials were completed; however, the results were available in ClinicalTrials.gov for only 44 (31.9%) trials. North America was the most frequently identified study location (52.9%), followed by Europe (30.4%) and Asia (11.6%). Seventy-one percent trials focused on viral pathogens, followed by bacterial pathogens (22.5%). A total of 84.1% trials were prevention oriented. A total of 84.1% trials used randomization, 73.2% trials used parallel assignment, and 64.5% used masking. Eighty-six trials were industry-funded and 52 were non-industry-funded. Industry-funded trials had higher percentages than non-industry-funded trials in available results, prevention trial, and phase 2 and phase 3 trial, and lager sample size trial. One hundred eleven trials were vaccine trials and 27 trials were non-vaccine trials. Vaccine trials had higher percentages than non-vaccine trials in available results, leading industry sponsor and viral etiology.

Conclusions: The current study is the first study of the landscape of interventional clinical trials for infectious diseases in old populations registered in ClinicalTrials.gov, providing the

basis for treatment and prevention of infectious diseases in old populations. Trials in this field are still relatively lacking, and additional and better trials are needed.

Keywords: infectious disease, old population, clinical trial, intervention, ClinicalTrials.gov

INTRODUCTION

Infectious diseases in old populations became an increasingly important global issue (Liang, 2016). The declining immune system, weakened anatomic and physiologic defenses against pathogens, and medical comorbidities increases the risk for infections in old populations (Liang, 2016), and results in a high rate of morbidity and mortality in old populations (Gavazzi and Krause, 2002). Since 1980, influenza and pneumonia ranked among the top 10 causes of death in patients aged over 65 years (Giarratano et al., 2018). Certain optimum drug therapies in younger adults might not be suitable in old populations owing to altered pharmacokinetics and pharmacodynamics (Gavazzi and Krause, 2002). Moreover, increased multidrug-resistant infections occurred in old populations (Denkinger et al., 2013). Thus, effective prevention and treatment strategies based on evidence are critically needed.

Evidence-based practice in old populations relies on clinical trials that were rigorous, transparent, and devoid of bias (Vidaeff et al., 2016; Alarcon-Ruiz et al., 2019). Clinical trials provided evidence for clinical practice and were widely regarded as the most crucial evidence source of efficacy and safety (Ruff et al., 2014). Thus, exploring clinical trials, especially analyzing registered clinical trials, were hot spots to help future clinical practice. Several studies provided comprehensive details about registered trials in several fields (Pasquali et al., 2012; Menezes et al., 2013; Hill et al., 2014; Chen et al., 2018); however, there is paucity of published works on the subject of intervention of infectious diseases in old populations. ClinicalTrials.gov (Califf et al., 2012) provides publicly accessible data of registered clinical trials, affords the most comprehensive source for identifying and tracking completed or ongoing trials, and is the best way to explore the characteristics of registered trials in particular fields (Pasquali et al., 2012; Menezes et al., 2013; Hill et al., 2014; Chen et al., 2018). Thus, we performed the current cross-sectional study to investigate the characteristic of registered trials regarding intervention against infectious diseases in old populations.

METHODS

Reporting Guideline

This was a cross-sectional study, and it was reported according to the reporting guideline STROBE (Zeng et al., 2015).

Searching of Registered Trials

ClinicalTrials.gov was used to identify registered trials on the intervention of infectious diseases in old populations. We used the advanced search function with the search terms, including viral OR bacterial OR fungal OR parasitic OR infectious disease on May 8th, 2019.

Screening Search Trials

Searched results were screened based on the study types as classified by the ClinicalTrials.gov. We used the age field as a filter; we included trials designed specifically for adults over age 65 years. Next, we manually reviewed all trials and selected trials regarding intervention of infectious diseases. Trials regarding non-infectious diseases were all excluded.

Data Extraction

The following information was extracted: NCT number, title, status, availability of the study results, conditions, interventions, primary funding, primary sponsor, trial phase, enrollment, study design (allocation, intervention model, masking, primary purpose), start date, and location.

Statistical Analysis

Descriptive analyses were used. Primary funding were classified as industry, the National Institutes of Health (NIH), or other funding. The primary sponsors were classified as university, hospital, industry, or other sponsor. Categorical data were reported as frequency and percentage. Continuous variables were reported as median and interquartile range. We excluded missing data from calculations. The differences between counts of categorical variables using the chi-square test or Fisher exact test. All analyses were performed using the SPSS19.0 software. All P values of less than 0.05 were taken to be statistically significant.

RESULTS

Screening and Included Trials

In the initial search, we identified 33 178 registered trials on ClinicalTrials.gov. After excluding duplicated trials and trials with participants younger than 65 years old, 223 trials remained. After excluding non-interventional trials, we finally identified 138 trials focused on intervention of infectious diseases in old populations (**Figure 1**).

General Characteristics of Included Trials

The characteristics of included trials is shown in **Table 1**. Twenty-three trials were started before 2007, 50 trials were started between 2007 and 2012, and 49 trials were started between 2012 and 2017. The status of most trials (N=105, 76.1%) was completed. However, only 31.9% of trials had available results in the database. The lead sponsors were as follows: industry (66.7%), university (14.5%), and hospital (10.1%). North America was the most frequently identified

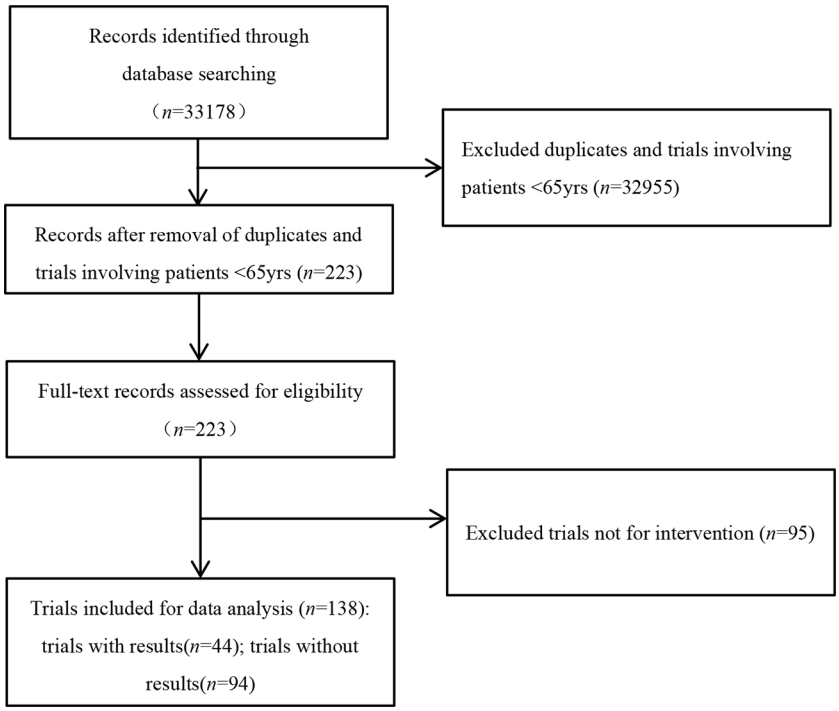

FIGURE 1 | Flowchart of selection trials.

study location (52.9%), followed by Europe (30.4%) and Asia (11.6%). The top four most commonly identified countries were the United States (N=68), Belgium (N=12), Italy (N=7), and Japan (N=7). Most trials were focused on viral pathogens (71.0%), followed by bacterial pathogens (22.5%).

Study Designs of Included Trials

Study designs of included trials are shown in **Table 2**. Most trials were for prevention, and only 10 trials were for treatment. Randomization was commonly used. The most frequently used intervention models were parallel assignment (73.2%) and single group assignment (21.0%). More than half of the trials were masked, and nearly a quarter of the trials involved quadruple masking. Phases of trials were as follows: phase 1 (13.0%), phase 2 (29.7%), phase 3 (21.7%), and phase 4 (23.2%). The estimated median enrollment was 242 participants (interquartile range, 84.5–821.5 participants). Forty-nine trials (35.5%) recruited more than 500 participants, and 25 trials (18.1%) recruited more than 1,000 individuals; another five trials recruited more than 10,000 participants.

Trials' Characteristics by Funding Source

Trials were most funded by industry (N=86, 62.3%). Comparison results are shown in **Table 3**. Industry-funded trials were mostly started during 2007–2012 whereas non-industry-funded trials mostly began during 2012–2017. Only 13.5% of non-industry-funded trials had available results, compared with 43.0% of industry-funded trials. Industry-funded studies were more focused on preventative interventions than non-industry-funded studies (91.9% *vs.* 71.2%). Parallel assignment (70.9%) and single group assignment (23.3%) were the most frequently used intervention models for industry-funded trials. Parallel assignment (76.9%) and sequential assignment (17.3%) were the most frequently used intervention models for non-industry-funded trials. More non-industry-funded trials were in phase 4 (40.4%), and only 12.8% of industry-funded trials were in phase 4. Industry-funded trials had larger enrollment than non-industry-funded trials. Microbial etiology, allocation, and masking were almost similar. Overall, compared with non-industry funded trials, industry-funded trials had higher percentages of available results, prevention trials, and phase 2 and phase 3 trials, and more lager sample size studies.

Trials' Characteristics by Vaccine Intervention

The trial characteristics of vaccine trials and non-vaccine trials are presented in **Table 4**. A total of 80.4% (N=111) of trials focused on vaccines. Among them, 78 trials investigated influenza vaccines, 16 trials investigated vaccines for pneumococcal diseases, and 17 trials investigated vaccines for other diseases, including herpes zoster, *C. difficile*-associated disease, tetanus, diphtheria, and Japanese encephalitis. Non-vaccine trials included antimicrobial trials (N=9), vitamin trials (N=5), probiotics trials (N=6), and other trials (N=7). A total of 53.8% non-vaccine trials were prevention-focused, and 34.6% trials were treatment-focused. Vaccine-related trials mostly

TABLE 1 | Characteristics of all included trials.

Variable	Subgroup	N (%)
Year		
	Prior to 2007	23 (16.7%)
	2007–2012	50 (36.2%)
	2012–2017	49 (35.5%)
	2017–now	16 (11.6%)
Status		
	Active, not recruiting	13 (9.4%)
	Completed	105 (76.1%)
	Recruiting	8 (5.8%)
	Suspended	1 (0.7%)
	Terminated	3 (2.2%)
	Unknown status	6 (4.3%)
	Withdrawn	2 (1.4%)
Study results		
	Has results	44 (31.9%)
	No results available	94 (68.1%)
Lead sponsor		
	University	20 (14.5%)
	Hospital	14 (10.1%)
	Industry	92 (66.7%)
	Other	12 (8.7%)
Funded by		
	Industry	86 (62.3%)
	NIH	6 (4.3%)
	Other	46 (33.3%)
Locations		
	Asia	16 (11.6%)
	Europe	42 (30.4%)
	North America	73 (52.9%)
	Oceania	5 (3.6%)
	South America	2 (1.4%)
Microbial etiology		
	Viral	98 (71.0%)
	Bacterial	31 (22.5%)
	Parasite	1 (0.7%)
	Unknown	8 (5.8%)
Participants		
	<=1000	113 (81.9%)
	1,000–10,000	20 (14.5%)
	>10,000	5 (3.6%)

TABLE 2 | Study design of all included trials.

Variable	Subgroup	N(%)
Primary purpose		
	Prevention	116 (84.1%)
	Treatment	10 (7.2%)
	Other	12 (8.7%)
Allocation		
	Randomized	116 (84.1%)
	Non-randomized	3 (2.2%)
	Unknown	19 (13.8%)
Intervention model		
	Crossover assignment	2 (1.4%)
	Factorial assignment	3 (2.2%)
	Parallel assignment	101 (73.2%)
	Sequential assignment	2 (1.4%)
	Single group assignment	29 (21.0%)
	Unknown	1 (0.7%)
Masking		
	Single	18 (13.0%)
	Double	25 (18.1%)
	Triple	12 (8.7%)
	Quadruple	34 (24.6%)
	None (open label)	48 (34.8%)
	Unknown	1 (0.7%)
Phases		
	Phase 1	18 (13.0%)
	Phase 1|phase 2	7 (5.1%)
	Phase 2	41 (29.7%)
	Phase 3	30 (21.7%)
	Phase 4	32 (23.2%)
	Not applicable	10 (7.2%)
Enrollment		
	<=50	18 (13.0%)
	50–100	23 (16.7%)
	100–500	47 (34.1%)
	>=500	49 (35.5%)
	Unknown	1 (0.7%)

began during 2007–2012 and non-vaccine trials mostly began during 2012–2017. A total of 36.9% vaccine trials had available results, while only 11.1% non-vaccine trials had available results. Trials tended to be larger in vaccine trials than non-vaccine trials. The industry was the primary lead sponsor for vaccine trials, and university was the lead sponsor for non-vaccine trials. Overall, compare with non-vaccine trials, vaccine trials had higher percentages of available study results, leading industry sponsor and viral etiology studies.

Trial Characteristics With Available Results

Among the 138 trials, 44 trials reported results on website and 94 trials did not. Among the 44 trials, 22 trials published 28 peer-reviewed papers. The summarized characteristics of the 22 published trials are shown in **Table 5**, and the details of the 22 published trials are shown in **Supplement Table A**. Two trials started before 2007, 13 trials began during 2007–2012, seven trials began during 2012–2017. Lead sponsors of trials were as follows: industry (72.7%), university (13.6%), and hospital (9.1%). The locations of countries were USA (86.4%), followed by Japan (9.1%) and Netherlands (1.0%). Fourteen trials were for viral pathogens (63.6%). Randomization (90.9%) was commonly used. More than half of trials were masked, and eight trials involved quadruple masking. Most trials were phase 3 (36.4%) and phase 4 (31.8%). Eleven trials (50.0%) recruited more than 500 participants, and eight trials (36.4%) recruited 100–500 participants, and the other three trials recruited less than 100 participants.

DISCUSSION

Clinical trials play important roles in clinical practice and decision-making (Ruff et al., 2014). Treatment of infectious diseases in old populations to reduce morbidity and mortality depends on well-designed trials. Interventional clinical trials for infectious diseases in old population have arisen much attention in recent years (Madan et al., 2017; Frey et al., 2019), however, little is known about the characteristics of registered clinical trials regarding this field. To the best of our knowledge, our study is the first to report registered trials in such field, and the results will

TABLE 3 | Characteristics and study design of trials according to the primary funding source.

Variable	Subgroup	Industry-funded (N=86)	Non-industry-funded (N=52)	χ^2/ Fisher	*P* value
Year				12.993	0.005
	Prior to 2007	13 (15.1%)	10 (19.2%)		
	2007–2012	39 (45.3%)	11 (21.2%)		
	2012–2017	22 (25.6%)	27 (51.9%)		
	2017–now	12 (14.0%)	4 (7.7%)		
Status				8.031*	0.178
	Active, not recruiting	5 (5.8%)	8 (15.4%)		
	Completed	71 (82.6%)	34 (65.4%)		
	Recruiting	3 (3.5%)	5 (9.6%)		
	Suspended	1 (1.2%)	0 (0.0%)		
	Terminated	2 (2.3%)	1 (1.9%)		
	Unknown status	3 (3.5%)	3 (5.8%)		
	Withdrawn	1 (1.2%)	1 (1.9%)		
Study results					
	Has results	37 (43.0%)	7 (13.5%)		
	No results available	49 (57.0%)	45 (86.5%)		
Lead sponsor				127.873*	<0.001
	University	0 (0.0%)	20 (38.5%)		
	Hospital	0 (0.0%)	14 (26.9%)		
	Industry	86 (100.0%)	6 (11.5%)		
	Other	0 (0.0%)	12 (23.1%)		
Primary purpose				12.512*	0.001
	Prevention	79 (91.9%)	37 (71.2%)		
	Treatment	5 (5.8%)	5 (9.6%)		
	Other	2 (2.3%)	10 (19.2%)		
Allocation				1.429*	0.592
	Randomized	72 (83.7%)	44 (84.6%)		
	Non-randomized	1 (1.2%)	2 (3.8%)		
	Unknown	13 (15.1%)	6 (11.5%)		
Intervention model				30.102*	<0.001
	Crossover assignment	0 (0.0%)	2 (3.8%)		
	Factorial assignment	3 (3.5%)	0 (0.0%)		
	Parallel assignment	61 (70.9%)	40 (76.9%)		
	Sequential assignment	2 (2.3%)	9 (17.3%)		
	Single group assignment	20 (23.3%)	0 (0.0%)		
	Unknown	0 (0.0%)	1 (0.9%)		
Masking				8.101*	0.127
	Single	7 (8.1%)	11 (21.2%)		
	Double	14 (16.3%)	11 (21.2%)		
	Triple	9 (10.5%)	3 (5.8%)		
	Quadruple	24 (27.9%)	10 (19.2%)		
	None (open label)	32 (37.2%)	16 (30.8%)		
	Unknown	0 (0.0%)	1 (1.9%)		
Phases				44.375*	<0.001
	Phase 1	12 (14.0%)	6 (11.5%)		
	Phase 1|phase 2	3 (3.5%)	4 (7.7%)		
	Phase 2	34 (49.5%)	7 (13.5%)		
	Phase 3	26 (30.2%)	4 (7.7%)		
	Phase 4	11 (12.8%)	21 (40.4%)		
	Not applicable	0 (0.0%)	10 (19.2%)		
Enrollment				13.608*	0.005
	<=50	6 (7.0%)	12 (23.1%)		
	50–100	17 (19.8%)	6 (11.5%)		
	100–500	26 (30.2%)	21 (40.4%)		
	>=500	37 (43.0%)	12 (23.1%)		
	Unknown	0 (0.0%)	1 (1.9%)		

*Fisher exact test.

provide the basis of the characteristics of trials design, location, and sponsor in this field.

Our study found that the number of trials explicitly designed to investigate interventions for old populations with infectious diseases was relatively small. Thus, evidence for old populations was lacking, and only a few trials were explicitly designed for this population (Carroll and Zajicek, 2011; Bellera et al., 2013; Banzi et al., 2016; White et al., 2019). It is important to address that old populations are likely to be excluded from infectious disease trials than non-infectious disease trials (Goswami et al., 2013). The reason might be that it was difficult to enroll enough old patients in trials, or low drug profit margins (Goswami et al., 2013). With the accelerating of ageing progress, it is urgent to start more trials in old populations to provide evidence for clinical practice.

In our study, most trials were focusing on prevention strategies (Goswami et al., 2013), which was quite different from trials in younger populations. Vaccinations for influenza and pneumonia were most frequently assessed. The overrepresentation of vaccine trials was influenced by the fact that most trials were performed in the US. Influenza and pneumonia were the most common infectious diseases in the US, and vaccination programs form part of routine clinical care in that country (Liang, 2016). Compared with high-income countries, old populations in low- and middle-income countries suffered the heavier burden of infectious diseases (Prince et al., 2015), including diarrhea, HIV/AIDS, tuberculosis, and malaria; however, there were not so many trials from low- and middle-income countries. Thus, it is suggested that high-income countries help low- and middle-income countries to conduct more trials. Another reason may be that trials from low- and middle-income countries are registered in other registries.

In our study, although 18.1% of trials were in phase 1 or phase 1/phase 2, only a few of them investigated novel drugs, despite increasing antimicrobial resistance. In addition, well-designed and adequately conducted trials were regarded as the best source of evidence. Randomization, blinding, and an appropriate patient population were the hallmarks of high-quality trials (Zwierzyna et al., 2018). In our study, most trials were randomized, masked, parallel assignment, and had a large enrollment, suggesting good quality of the included trials.

TABLE 4 | Characteristics of vaccine and non-vaccine trials.

Variable	Subgroup	Vaccine (N=111)	Non-vaccine (N=27)	χ^2/ Fisher	*P* value
Year				2.803*	0.421
	Prior to 2007	17 (15.3%)	6 (22.2%)		
	2007–2012	43 (38.7%)	7 (25.9%)		
	2012–2017	37 (33.3%)	12 (44.4%)		
	2017–now	14 (12.6%)	2 (7.4%)		
Status				3.456*	0.751
	Active, not recruiting	11 (9.9%)	2 (7.4%)		
	Completed	85 (76.6%)	20 (74.1%)		
	Recruiting	6 (5.4%)	2 (7.4%)		
	Suspended	1 (0.9%)	0 (0.0%)		
	Terminated	2 (1.8%)	1 (3.7%)		
	Unknown status	5 (4.5%)	1 (3.7%)		
	Withdrawn	1 (0.9%)	1 (3.7%)		
Study results				6.670	0.011
	Has results	41 (36.9%)	3 (11.1%)		
	No results available	70 (63.1%)	24 (88.9%)		
Lead sponsor				24.400*	<0.001
	University	10 (9.0%)	10 (37.0%)		
	Hospital	7 (6.3%)	7 (25.9%)		
	Industry	84 (75.7%)	8 (29.6%)		
	Other	10 (9.0%)	2 (7.4%)		
Funded by				22.864*	<0.001
	Industry	79 (71.2%)	7 (25.9%)		
	NIH	6 (5.4%)	0 (0.0%)		
	Other	26 (23.4%)	20 (74.1%)		
Locations				7.695*	0.078
	Asia	15 (13.5%)	1 (3.7%)		
	Europe	33 (29.7%)	9 (33.3%)		
	North America	59 (53.2%)	14 (51.9%)		
	Oceania	4 (3.6%)	1 (3.7%)		
	South America	0 (0.0%)	2 (7.4%)		
Microbial etiology				32.107*	<0.001
	Viral	87 (78.4%)	11 (40.7%)		
	Bacterial	24 (21.6%)	7 (25.9%)		
	Parasite	0 (0.0%)	1 (3.7%)		
	Unknown	0 (0.0%)	8 (29.6%)		

**Fisher exact test.*

TABLE 5 | Characteristics of the 22 trials published results.

Variable	Subgroup	N (%)
Year		
	Prior to 2007	2 (9.1%)
	2007–2012	13 (59.1%)
	2012–2017	7 (31.8%)
Lead sponsor		
	University	3 (13.6%)
	Hospital	2 (9.1%)
	Industry	16 (72.7%)
	Other	1 (4.5%)
Funded by		
	Industry	16 (72.7%)
	Other	6 (27.3%)
Locations		
	Japan	2 (9.1%)
	Netherlands	1 (4.5%)
	USA	19 (86.4%)
Microbial etiology		
	Viral	14 (63.6%)
	Bacterial	7 (31.8%)
	Unknown	1 (4.5%)
Primary purpose		
	Prevention	21 (95.5%)
	Other	1 (4.5%)
Allocation		
	Randomized	20 (90.9%)
	Unknown	2 (9.1%)
Intervention model		
	Parallel assignment	20 (90.9%)
	Single group assignment	2 (9.1%)
Masking		
	Single	3 (13.6%)
	Double	1 (4.5%)
	Triple	2 (9.1%)
	Quadruple	8 (36.4%)
	None (open label)	8 (36.4%)
Phases		
	Phase 1	1 (4.5%)
	Phase 2	5 (22.7%)
	Phase 3	8 (36.4%)
	Phase 4	7 (31.8%)
	Not Applicable	1 (4.5%)
Enrollment		
	<=50	2 (9.1%)
	50–100	1 (4.5%)
	100–500	8 (36.4%)
	>=500	11 (50.0%)

Providing trials' results was more and more important. In our study, although 76.1% trials were completed, only 31.9% provided results on the database, the low percentage of available results was consistent with results in previous study (Zwierzyna et al., 2018). In addition, there was an increasing concern of industry role in trial design, conduct, and funding (Johnson and Stricker, 2010). A total of 62.3% trials were funded by industry, which was much more than drug control and prevention of ventilator-associated pneumonia (Chen et al., 2018), suggesting the lack of other sources of funding in interventional clinical trials on infectious diseases in old populations. Study designs between industry-funded trials and non-industry-funded infectious disease trials were similar. Compared with non-industry-funded trials, industry-funded trials had a higher proportion of trials with available results and a larger enrollment. Most trials were funded by large pharmaceutical companies, which had better financial and organizational resources and more experts in conducting trials (Laterre and Francois, 2015). Our study revealed that vaccine trials had higher percentages of study results, leading industry sponsor and viral etiology trials, which suggested more treatment trials should be performed in this field.

There are several limitations to our study. First, ClinicalTrials.gov is the largest trial registry in the world, containing more than

80% of all trials in the World Health Organization International Clinical Trials Registry Platform. However, we could not exclude the possibility that some trials are registered in other trial registries. Second, our study is only a cross-sectional study, which limits our further analysis of potential influential factors. Third, as ClinicalTrials.gov is not designed to support for data analysis, it limits us to perform data synthesis; with the development of technology, researches can be combined by using data from different trials for the same topic.

In conclusion, this study provides useful information about registered interventional clinical trials on infectious diseases in old populations; this analysis will potentially help stakeholders, including investigators, academic centers, and industry to take future decisions regarding the conduct of clinical trials in this population. Additional and better trials are needed to provide more evidence.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

YZ designed the study. LC searched the data, analyzed the data, and drafted the manuscript. MW performed the initial search. YZ, LC, and YY revised the manuscript. JS helped to prepare the study and applied the supported grant. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00942/full#supplementary-material

REFERENCES

Alarcon-Ruiz, C. A., Roque-Roque, J. S., Heredia, P., Gómez-Briceño, A. R., and Quispe, A. M. (2019). Twenty-two years' experience registering trials in a low-middle income country: The Peruvian Clinical Trial Registry. *J. Evid. Based Med.* 12 (3), 187–193. doi: 10.1111/jebm.12354.

Banzi, R., Camaioni, P., Tettamanti, M., Bertele, V., and Lucca, U. (2016). Older patients are still under-represented in clinical trials of Alzheimer's disease. *Alzheimers Res. Ther.* 8, 32. doi: 10.1186/s13195-13016-10201-13192

Bellera, C., Praud, D., Petit-Moneger, A., McKelvie-Sebileau, P., Soubeyran, P., and Mathoulin-Pelissier, S. (2013). Barriers to inclusion of older adults in randomised controlled clinical trials on Non-Hodgkin's lymphoma: a systematic review. *Cancer Treat Rev.* 39 (7), 812–817. doi: 10.1016/j.ctrv.2013.1001.1007

Califf, R. M., Zarin, D. A., Kramer, J. M., Sherman, R. E., Aberle, L. H., and Tasneem, A. (2012). Characteristics of clinical trials registered in ClinicalTrials.gov 2007-2010. *JAMA* 307 (17), 1838–1847. doi: 10.1001/jama.2012.3424.

Carroll, C. B., and Zajicek, J. P. (2011). Designing clinical trials in older people. *Maturitas* 68 (4), 337–341. doi: 10.1016/j.maturitas.2011.1002.1002

Chen, L., Su, Y., Quan, L., Zhang, Y., and Du, L. (2018). Clinical Trials Focusing on Drug Control and Prevention of Ventilator-Associated Pneumonia: A Comprehensive Analysis of Trials Registered on ClinicalTrials.gov. *Front. Pharmacol.* 9, 1574. doi: 10.3389/fphar.2018.01574

Denkinger, C. M., Grant, A. D., Denkinger, M., Gautam, S., and D'Agata, E. M. (2013). Increased multi-drug resistance among the elderly on admission to the hospital–a 12-year surveillance study. *Arch. Gerontol. Geriatr.* 56 (1), 227–230. doi: 10.1016/j.archger.2012.05.006.

Frey, S. E., Shakib, S., Chanthavanich, P., Richmond, P., Smith, T., Tantawichien, T., et al. (2019). Safety and Immunogenicity of MF59-Adjuvanted Cell Culture-Derived A/H5N1 Subunit Influenza Virus Vaccine: Dose-Finding Clinical Trials in Adults and the Elderly. *Open Forum Infect. Dis.* 6 (4), ofz107. doi: 10.1093/ofid/ofz107.

Gavazzi, G., and Krause, K. H. (2002). Ageing and infection. *Lancet Infect. Dis.* 2 (11), 659–666. doi: 10.1016/S1473-3099(02)00437-1

Giarratano, A., Green, S. E., and Nicolau, D. P. (2018). Review of antimicrobial use and considerations in the elderly population. *Clin. Interv. Aging* 13, 657–667. doi: 10.2147/CIA.S133640

Goswami, N. D., Pfeiffer, C. D., Horton, J. R., Chiswell, K., Tasneem, A., and Tsalik, E. L. (2013). The state of infectious diseases clinical trials: a systematic review of ClinicalTrials.gov. *PloS One* 8 (10), e77086. doi: 10.71371/journal.pone.0077086

Hill, K. D., Chiswell, K., Califf, R. M., Pearson, G., and Li, J. S. (2014). Characteristics of pediatric cardiovascular clinical trials registered on ClinicalTrials.gov. *Am. Heart J.* 167 (6), 921–929.e922. doi: 10.1016/j.ahj.2014.1002.1002

Johnson, L., and Stricker, R. B. (2010). The Infectious Diseases Society of America Lyme guidelines: a cautionary tale about the development of clinical practice guidelines. *Philos. Ethics Humanit. Med.* 5, 9. doi: 10.1186/1747-5341-1185-1189.

Laterre, P. F., and Francois, B. (2015). Strengths and limitations of industry vs. academic randomized controlled trials. *Clin. Microbiol. Infect.* 21 (10), 906–909. doi: 10.1016/j.cmi.2015.1007.1004

Liang, S. Y. (2016). Sepsis and Other Infectious Disease Emergencies in the Elderly. *Emerg. Med. Clin. North Am.* 34 (3), 501–522. doi: 10.1016/j.emc.2016.1004.1005.

Madan, A., Ferguson, M., Rheault, P., Seiden, D., Toma, A., Friel, D., et al. (2017). Immunogenicity and safety of an AS03-adjuvanted H7N1 vaccine in adults 65years of age and older: A phase II, observer-blind, randomized, controlled trial. *Vaccine* 35 (15), 1865–1872. doi: 10.1016/j.vaccine.2017.02.057.

Menezes, A. S., Barnes, A., Scheer, A. S., Martel, G., Moloo, H., Boushey, R. P., et al. (2013). Clinical research in surgical oncology: an analysis of ClinicalTrials.gov. *Ann. Surg. Oncol.* 20 (12), 3725–3731. doi: 10.1245/s10434-10013-13054-y

Pasquali, S. K., Lam, W. K., Chiswell, K., Kemper, A. R., and Li, J. S. (2012). Status of the pediatric clinical trials enterprise: an analysis of the US ClinicalTrials.gov registry. *Pediatrics* 130 (5), e1269–e1277. doi: 10.1542/peds.2011-3565

Prince, M. J., Wu, F., Guo, Y., Gutierrez Robledo, L. M., O'Donnell, M., Sullivan, R., et al. (2015). The burden of disease in older people and implications for health policy and practice. *Lancet* 385 (9967), 549–562. doi: 10.1016/S0140-6736(1014)61347-61347

Ruff, C. T., Giugliano, R. P., Braunwald, E., Hoffman, E. B., Deenadayalu, N., Ezekowitz, M. D., et al. (2014). Comparison of the efficacy and safety of new oral anticoagulants with warfarin in patients with atrial fibrillation: a meta-analysis of randomised trials. *Lancet* 383 (9921), 955–962. doi: 10.1016/S0140-6736(13)62343-0.

Vidaeff, A. C., Saade, G. R., and Belfort, M. A. (2016). Interpreting a randomized trial report: evidence-based practice for the clinician. *J. Matern. Fetal. Neonatal. Med.* 29 (16), 2640–2644. doi: 10.3109/14767058.2015.1095882.

White, M. N., Dotan, E., Catalano, P. J., Cardin, D. B., and Berlin, J. D. (2019). Advanced pancreatic cancer clinical trials: The continued underrepresentation of older patients. *J. Geriatr. Oncol.* 10 (4), 540–546. doi: 10.1016/j.jgo.2018.1011.1001

Zeng, X., Zhang, Y., Kwong, J. S., Zhang, C., Li, S., Sun, F., et al. (2015). The methodological quality assessment tools for preclinical and clinical studies, systematic review and meta-analysis, and clinical practice guideline: a systematic review. *J. Evid. Based Med.* 8 (1), 2–10. doi: 10.1111/jebm.12141.

Zwierzyna, M., Davies, M., Hingorani, A. D., and Hunter, J. (2018). Clinical trial design and dissemination: comprehensive analysis of clinicaltrials.gov and PubMed data since 2005. *BMJ* 361, k2130. doi: 10.1136/bmj.k2130

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Coronavirus Disease 2019 Related Clinical Studies: A Cross-Sectional Analysis

Lin-Lu Ma[1†], Xuan Yin[2†], Bing-Hui Li[1,3], Jia-Yu Yang[1,4], Ying-Hui Jin[1], Di Huang[1], Tong Deng[3], Yun-Yun Wang[1], Xue-Qun Ren[3], Jianguang Ji[5*] and Xian-Tao Zeng[1*]*

[1] Center for Evidence-Based and Translational Medicine, Zhongnan Hospital of Wuhan University, Wuhan, China, [2] Department of Rehabilitation, Taihe Hospital, Hubei University of Medicine, Shiyan, China, [3] Institutes of Evidence-Based Medicine and Knowledge Translation, Henan University, Kaifeng, China, [4] Administrative Office of Hospital Director, Zhongnan Hospital of Wuhan University, Wuhan, China, [5] Center for Primary Health Care Research, Lund University/Region Skåne, Malmö, Sweden

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UCB Pharma, Belgium

***Correspondence:**
Xue-Qun Ren
renxuequn001@163.com
Jianguang Ji
jianguang.ji@med.lu.se
Xian-Tao Zeng
zengxiantao1128@163.com

[†]These authors share first authorship

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Objective: The quality and rationality of many recently registered clinical studies related to coronavirus disease 2019 (COVID-19) needs to be assessed. Hence, this study aims to evaluate the current status of COVID-19 related registered clinical trial.

Methods: We did an electronic search of COVID-19 related clinical studies registered between December 1, 2019 and February 21, 2020 (updated to May 28, 2020) from the *ClinicalTrials.gov*, and collected registration information, study details, recruitment status, characteristics of the subjects, and relevant information about the trial implementation process.

Results: A total of 1,706 studies were included 10.0% of which (n=171) were from France, 943 (55.3%) used an interventional design, and 600 (35.2%) used an observational design. Most of studies (73.6%) aimed to recruit fewer than 500 people. Interferon was the main prevention program, and antiviral drugs were the main treatment program. Hydroxychloroquine and chloroquine (230/943, 24.4%) were widely studied. Some registered clinical trials are incomplete in content, and 37.4% of the 1,706 studies may have had insufficient sample size.

Conclusion: The quality of COVID-19 related studies needs to be improved by strengthening the registration process and improving the quality of clinical study protocols so that these clinical studies can provide high-quality clinical evidence related to COVID-19.

Keywords: coronavirus disease 2019, SARS-CoV-2, clinical trial, registration, *ClinicalTrials.gov*

INTRODUCTION

COVID-19, which broke out at the beginning of 2020, has spread rapidly (Zhou P. et al., 2020). Its clinical manifestations are very similar to Severe Acute Respiratory Syndrome (SARS). In severe cases, patients may go on to develop acute respiratory distress syndrome (ARDS). Patients with severe COVID-19 need intensive care to decrease mortality (Huang et al., 2020). As of July 13, 2020,

there have been more than 12.8 million confirmed cases and 568,000 deaths globally (Johns Hopkins University, 2020).

COVID-19 is an emerging infectious disease for which, there is no specific treatment to date. Healthcare professionals have only been able to alleviate patients' symptoms based on their experience (Jin et al., 2020) as up to now they have had insufficient knowledge of this disease. Hence, randomized clinical trials (RCTs) are necessary to verify the safety and effectiveness of the proposed drugs. Many scientists and clinicians have conducted clinical investigations, diagnostic accuracy tests, and treatment evaluations to understand the progress of COVID-19 and to improve clinical diagnosis and treatment. It is thus essential to evaluate the rationality and the potential value of proposed clinical trials because so many studies have emerged in such a short period and some of them might lack scientific value. Therefore, we performed this survey in order to have a comprehensive understanding of the current clinical trials related to COVID-19.

METHODS

This study analyzed the characteristics of the clinical studies of COVID-19 registered in *ClinicalTrials.gov* (https://clinicaltrials.gov/) between December 1, 2019 and February 21, 2020 (updated to May 28, 2020). All COVID-19 related studies, including etiology, risk factors, prevention, diagnosis, treatment, prognosis, and psychology were included. The search terms were: 2019-nCoV, 2019 novel coronavirus, novel coronavirus pneumonia, COVID-19, coronavirus disease 2019, SARS-CoV-2.

We extracted the following information from registered studies: registration number, registration date, registration title, primary sponsor, funding source, study type, study phase, study objectives, study design, length of the study, intervention, countries of recruitment and research settings, recruiting status, allocation, sample size, participant age, gender, masking, the time and method of sharing individual participant data (IPD), data management committee.

Descriptive statistics were used to summarize the characteristics of all included clinical studies. Categorical variables were expressed as percentages and frequencies. All data were summarized using Microsoft Excel 2019.

RESULTS

General Characteristics of the Included Studies

A total of 1,706 studies were included. Among these clinical studies (**Table 1**), the first one was registered on January 23, 2020, and the number of trials registered daily subsequently increased, peaking at 51 in a single day (**Figure 1**). For the total study period, 73.8% studies (n = 1259) planned to continue for less than 12 months and 25.1% more than 12 months. Of them, 943 (55.3%) used an interventional design and 600 (35.2%) used an observational design. As for the recruitment status, 82 (4.8%) studies had completed recruitment, 922 (54.0%) were recruiting, and 683 (40.0%) had not yet started recruiting, while some others were terminated/withdrawn (n = 12, 0.7%) or suspended (n = 7, 0.4%). For sample sizes, most of them (n = 1255, 73.6%) aimed to recruit less than 500 participants, 6.5% (n = 111) recruited 100 to 499 participants, 18.9% recruited more than 1,000, and 1.1% (n = 18) studies did not specify the number of participants recruited. Almost all studies recruited both males and females (n = 1662, 97.4%), 83.1% studies (n = 1417) included adults and only 16.9% (n = 289) involved children.

TABLE 1 | General characteristics of the included studies.

	Number	Percentage
Total	1706	100%
Length of study time		
0 <L≤6m	816	47.8%
6m<L≤12m	443	26.0%
12m<L≤18m	154	9.0%
L>18m	274	16.1%
NA	19	1.1%
Study type		
Interventional	943	55.3%
Observational	600	35.2%
Diagnostic Test	145	8.5%
Expanded Access	18	1.1%
Recruitment status		
Recruiting	922	54.0%
Not yet recruiting	683	40.0%
Completed	82	4.8%
Terminated/Withdrawn	12	0.7%
Suspended	7	0.4%
Enrollment		
<100	638	37.4%
100–499	617	36.2%
500–999	111	6.5%
≥1000	322	18.9%
NA	18	1.1%
Sex/Gender		
Both	1662	97.4%
Only female	35	2.1%
Only male	7	0.4%
NA	2	0.1%
Ages		
Child	20	1.2%
Adult	1417	83.1%
Child and Adult	269	15.8%

NA, Not available. Child means a person under the age of 18.

Methodological Quality of the Included Studies

Among the 943 interventional studies, the primary purpose was treatment of the disease (n = 714, 75.7%). Seven hundred eighty-one (82.8%) were designed with at least two groups, most commonly parallel assignment (n = 717, 76.0%). Seven hundred twenty (76.4%) were randomized and 78 (8.3%) were non-randomized. More than 56.2% studies (n = 530) were open label, and only 33.0% being double, triple, or quad-masked. As for the 600 observational studies, 376 (62.7%) were cohort studies, and 377 (62.8%) were prospective design. For the 145 diagnostic studies, 32 studies (22.1%) focused on imaging studies, 36 studies (24.8%) focused on nucleic acid detection,

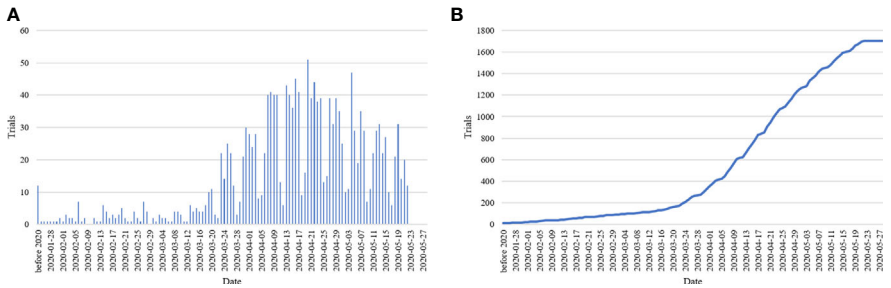

FIGURE 1 | Bar chart of registered studies per day **(A)** and cumulative sum chart of registered studies **(B)**.

and 15 studies (10.3%) focused on specific antibody. Details are shown in **Table 2**.

Detailed Characteristics of Included Studies

Of the 1,706 studies, 1,200 (70.3%) were initiated by researchers from hospitals, universities, or scientific research institutions; whereas a few (9.8%) were initiated by companies, and 338 (19.8%) were funded by others, such as individuals or community-based organizations. The highest number of studies were conducted in France (n = 171, 10.0%) and the second highest in the United States (n = 108, 6.3%). Of the 1,706 studies, only 33 studies (1.9%) were funded by National Institutes of Health (NIH) or U.S. Federal agencies, 255 (14.9%) were funded by pharmaceutical or device companies, and 83.1% were funded by others, such as individuals, universities, or community-based organizations. Six hundred ten (35.8%) clearly reported the existence of a data monitoring committee, and 192 (11.3%) had IPD sharing statement. Details are shown in **Table 3**.

Description of Drugs in the Included Interventional Studies

Among the 943 interventional studies, 416 studies (44.1%) explored the effectiveness and/or safety of drugs commonly used in preventing and treating COVID-19, such as hydroxychloroquine (HCQ), chloroquine (CQ), immunotherapy (including stem cell therapy, monoclonal antibody, immunoregulation), lopinavir/ritonavir, glucocorticoids, interferon, targeted therapy (Baricitinib, Ruxolitinib, Imatinib), favipiravir, and Remdesivir. In addition, 66 studies (7.0%) focused on convalescent plasma. Other interventions, such as dietary supplements, devices and behavioral programs, accounted for 48.9%. Details are shown in **Table 4**.

DISCUSSION

The COVID-19 epidemic is still raging around the world. Exploring the characteristics of registered clinical studies related to COVID-19 and clarifying the direction of further research can help reduce the potential disease burden of COVID-19 (Gupta et al., 2020). There was a cross-sectional study that reviewed the drug and plasma registration trials in March 2020, characterizing the scope, objectives and content of clinical studies (Mehta et al., 2020). With the rapid increase in registration research, the status of registration studies may also change. This survey conducted a comprehensive summary of COVID-19 related studies registered in the *ClinicalTrials.gov* as of May 28, 2020. Results showed that most studies with an interventional design were aimed at adult participants, and were conducted using multicenter, randomized, parallel assignments, and open-label methods. A systematic review showed that compared with adults, children with COVID-19 have a milder disease course, with better prognosis and extremely low mortality (Ludvigsson, 2020). As a result, only 16.9% of registered studies involved children. As a factor of disease outcomes (Hou et al., 2019), only 2.5% studies focus on the participants' gender. The included clinical studies involved disease prevention, diagnostic accuracy, drug treatment, medical devices, prognosis, as well as treatment of critical COVID-19. A number of these studies (n=638, 37.4%) may have had insufficient sample size.

Registration of COVID-19 related clinical studies is ongoing. The underlying methodological quality limitations of these clinical studies should be noted, such as lack of control group, insufficient sample size, or non-randomization, which might preclude drawing concrete conclusions (Bauchner and Fontanarosa, 2020; Ma et al., 2020). Our results found that nearly half of the registered trials did not exceed 6 months, and 37.4% of the registered trials recruited less than 100 people. The inclusion of less than 100 people does not automatically indicate that the study results are unreliable. Different studies need to estimate sample size according to outcomes. More studies are needed which use samples based on the estimated sample size. Insufficient or under-estimated sample size is a major shortcoming of the current clinical trials, which can cause false negative or false positive results, reduce credibility, and even have catastrophic consequences (Ruberg and Akacha, 2017). Therefore, although some studies had reported that some interventions may shorten intubation time, hospitalization time or reduce mortality; these findings did not represent the actual therapeutic effect of the drug (Gautret et al., 2020). The outbreak of the epidemic may pressurize researchers to quickly find targeted therapeutic drugs which are effective in the short term. However, if the length of the study was too short, it

TABLE 2 | Trials design data.

Study Type		Number	Percentage
Interventional	Total	943	100%
	Number of Arms		
	1	161	17.1%
	2	622	66.0%
	3	83	8.8%
	4	47	5.0%
	> 4	29	3.1%
	NA	1	0.1%
	Allocation		
	Randomized	720	76.4%
	Non-Randomized	78	8.3%
	NA	145	15.4%
	Intervention Model		
	Single Group Assignment	160	17.0%
	Parallel Assignment	717	76.0%
	Sequential Assignment	33	3.5%
	Factorial Assignment	12	1.3%
	Crossover Assignment	21	2.2%
	Masking (Blinding)		
	Open Label	530	56.2%
	Single	102	10.8%
	Double	109	11.6%
	Triple	66	7.0%
	Quadruple	136	14.4%
	Primary Purpose		
	Treatment	714	75.7%
	Prevention	124	13.1%
	Others	105	11.1%
	Phases		
	Early Phase 1	18	1.9%
	Phase 1	47	5.0%
	Phase 1|Phase 2	54	5.7%
	Phase 2	265	28.1%
	Phase 2|Phase 3	76	8.1%
	Phase 3	174	18.5%
	Phase 4	56	5.9%
	NA	253	26.8%
Observational	Total	600	100%
	Observational Model		
	Case-Only	52	8.7%
	Case-Control	74	12.3%
	Cohort	376	62.7%
	Other	98	16.3%
	Time Perspective		
	Retrospective	100	16.7%
	Prospective	377	62.8%
	Cross-Sectional	67	11.2%
	Other	56	9.3%
Diagnostic Test	Total	145	100%
	Imaging exams	32	22.1%
	nucleic acid detection	36	24.8%
	IgM/IgG	15	10.3%
	Other	62	42.8%

NA, Not available.

might preclude carrying out multiple follow-ups on the patients, and the long-term effect index of drug treatment cannot be obtained.

Our results found that of the intervention study 82.8% of the registered trials were designed for at least two groups, 76.4% were assigned randomly, 56.2% were open label, and 75.7% were mainly for treatment. Of the observational studies, most utilized cohorts (62.7%) and prospective (62.8%) designs. Randomization can largely avoid confusion and reduce selection bias in treatment comparison (Sessler and Imrey,

TABLE 3 | Sponsor, location, and data monitoring characteristics of the included studies.

	Number	Percentage
Total	1706	100%
Study Sponsor		
Hospital	593	34.8%
University	476	27.9%
Industry	168	9.8%
Research Institution	131	7.7%
Other	338	19.8%
Collaborators		
Has Collaborators	621	36.4%
No Collaborators	1085	63.6%
Places to recruit and conduct research		
France	171	10.0%
United States	108	6.3%
Italy	52	3.0%
China	43	2.5%
United Kingdom	35	2.1%
Spain	33	1.9%
Germany	29	1.7%
Egypt	25	1.5%
Other	154	9.0%
NA	1094	64.1%
Funder Type		
NIH	27	1.6%
U.S. Fed	6	0.4%
Industry	255	14.9%
Other	1418	83.1%
Data Monitoring Committee		
Has Data Monitoring Committee	610	35.8%
Not have Data Monitoring Committee	811	47.5%
NA	285	16.7%
IPD Sharing Statement		
Yes	192	11.3%
No	731	42.8%
Undecided	334	19.6%
NA	449	26.3%

NA, Not available.

TABLE 4 | Interventional clinical studies for COVID-19.

Interventions	Trials Number	Percentage
Total	943	100%
Hydroxychloroquine	157	16.6%
Hydroxychloroquine & Azithromycin	51	5.4%
Chloroquine	22	2.3%
Remdesivir	10	1.1%
Lopinavir/Ritonavir	32	3.4%
Favipiravir	16	1.7%
Interferon	23	2.4%
Glucocorticoid	29	3.1%
Immunity therapy	53	5.6%
Targeted Therapy	23	2.4%
Convalescent plasma	66	7.0%
Dietary Supplement	18	1.9%
Device	63	6.7%
Behavioral	47	5.0%
Other	333	35.3%

2015). However, RCTs often require large sample size, long research duration, incur high costs and may also be difficult to implement. At this time, adaptive trial design can usually be adopted (Bhatt and Mehta, 2016). However, it should be noted that observational research will be accompanied by some biases and limitations, and it is necessary to interpret the test results carefully (Shang et al., 2020). Besides, some of these studies did not have a control group or lack a real "control", which will limit the effective inferences that can be drawn. There is a need for rigorous design and attention to trial protocols for research drug management to discover the true efficacy of interventions (Bauchner and Fontanarosa, 2020).

More and more researchers realize that clinical trials need to be registered before the recruitment, and registration is beneficial for sharing clinical trial information and reducing publication bias (Aslam et al., 2013). It is understandable that clinical trials must be launched and implemented quickly due to the sudden COVID-19 epidemic; however, a properly designed clinical trial is still the core to provide scientific evidence and achieve clinical conclusions. Randomized controlled trials are considered to be the highest quality clinical research methods, and random sequence generation, blinding, and allocation concealment during the implementation of the study are critical to the success of the study (Schulz and Grimes, 2002b; Schulz and Grimes, 2002a; Sessler and Imrey, 2015). It is thus essential for clinical trials to be designed by a professional team to meet the requirements of a successful study before registration. An appropriate research design should be selected according to the research purpose, with sample size being estimated in advance, and timely submission of the research plan to the ethical committee to avoid deficiencies. At the time of registration, the person responsible for the registration should have a comprehensive understanding of the characteristics of the study protocol and clinical trial, so as not to cause confusion to other researchers due to the ambiguity of registration content, such as countries of location, presence or absence of data monitoring committee. We found some registered clinical trials have incomplete content. Therefore, clinical trial registration agencies should strengthen supervision of trial registration. After the study completion, collation and strict statistical analysis the researchers should upload the resulting data to the registration agency in a timely manner (Goldacre, 2017). IPD sharing helps to accelerate the conversion of clinical resources and promote scientific breakthroughs. Hence, we call on researchers to share IPD to promote transparency, so that effective conclusions drawn from trials can be quickly applied to control the epidemic, and to provide a basis for COVID-19 prevention and treatment.

COVID-19 is a new infectious disease, which has affected health insurance (Gheorghe et al., 2019), and its underlying mechanisms of transmission and pathogenesis are still being explored. High quality clinical studies are the basis of clinical practice guidelines, especially WHO's emergency guidelines (Norris et al., 2019). Some clinical trials focus on the prevention of COVID-19. It is widely believed that SARS-CoV-2 is transmitted through respiratory droplets and by close contact (Jin et al., 2020). Earlier studies have shown that masks are very effective for filtering influenza viruses (Zhou et al., 2018). However, there are no clinical study results that can prove that wearing masks can prevent COVID-19. A study has analyzed the pandemic trends and mitigation measures of COVID-19 in Wuhan, China, Italy, and New York City. Results showed that the difference between with and without facial masks represents the determinant of pandemic trends in the three epicenters. The authors thought that wearing a mask is the most effective way to prevent interpersonal transmission in public places (Zhang R. et al., 2020). In hospitals, healthcare professionals are at greater risk of exposure to SARS-CoV-2 than public. A multi-center RCT (Registration number: *NCT04296643*) from Canada is expected to recruit 576 nurses to compare and analyze the preventive effects of medical masks with N95 respirators on COVID-19. In addition, some clinical studies have focused on the preventive effects of drugs for COVID-19, such as CQ and HCQ. Chloroquine and hydroxychloroquine are both antimalarial drugs, and the mechanism of preventing and treating COVID-19 is not yet clear. Some researchers thought CQ and HCQ may confer antiviral effect at the pre-infection stages (Zhou D. et al., 2020). However, the possible cardiac side effects caused by the combination of CQ or HCQ and AZ, such as prolonged QT interval must be considered. Hence, clinical studies are needed to confirm the preventive effect of CQ or HCQ on COVID-19 (Registration number: *NCT04303507, NCT04334148*).

An accurate diagnosis is the fundamental prerequisite for efficient control of COVID-19. We included 145 clinical studies exploring the diagnosis of COVID-19. These diagnostic accuracy tests mainly focus on imaging examination, nucleic acid detection, and IgM/IgG. Detection of SARS-CoV-2 RNA by reverse-transcription polymerase chain reaction (RT-PCR) is the most commonly used to diagnose COVID-19. Early studies have shown that RT-PCR has relatively poor sensitivity, and false negative test results will miss some potential infected persons, which has a huge impact (Fang et al., 2020). Furthermore, the standard RT-PCR test takes about 3 h to complete. The cost of each test is about $10. The high cost per test may limit the number of tests (Esbin et al., 2020). Hence, researchers wanted to design some test kits in order to detect SARS-CoV-2 quickly and conveniently (Chu et al., 2020; Shirato et al., 2020; To et al., 2020; Yu et al., 2020). COVID-19 patients also have some typical computed tomography (CT) manifestations, such as ground glass opacities (Fang et al., 2020; Lu et al., 2020; Zhang J. J. et al., 2020). As a fast and effective method, CT can be used for auxiliary diagnosis. However, it should be noted that some patients may have atypical CT imaging manifestations (Jin et al., 2020; Lu et al., 2020; Wang W. G. et al., 2020). In addition, as the product of human immune system reaction to SARS-CoV-2, IgM/IgG can provide information about the course of the virus infection over time and provide the basis for the diagnosis of COVID-19. Some researchers have developed an IgM-IgG combined antibody test kit with a sensitivity of 88.66% and a specificity of 90.63%, but there were still false negative and false positive results (Li et al., 2020). The sensitivity and specificity of the IgM/IgG rapid diagnostic kit are currently being evaluated in some studies (*Registration number: NCT04346186, NCT04348864*).

Drug treatment is a very important part of the registration studies. Few drugs were used to treat COVID-19, such as CQ, HCQ, IFN, lopinavir/ritonavir, Oseltamivir, Umifenovir, dexamethasone. There is currently no clear evidence that these drugs are specific drugs for the treatment of COVID-19 other than dexamethasone (Gautret et al., 2020; RECOVERY Collaborative Group, 2020; Tang et al., 2020). The RECOVERY trial claims that dexamethasone can reduce the risk of death for patients on ventilators (RR 0.64; 95% CI, 0.51 to 0.81) and patients on oxygen (RR 0.82; 95% CI, 0.72 to 0.94) (RECOVERY Collaborative Group, 2020). The National Institutes of Health recommends the use of dexamethasone to treat COVID-19 patients who require supplemental oxygen in its guidelines (COVID-19 Treatment Guidelines Panel, 2020). As a new experimental broad-spectrum antiviral medication, Remdesivir is considered to be effective in inhibiting the replication of SARS coronavirus and MERS coronavirus. Two RCT studies showed that compared with placebo, the use of Remdesivir could shorten the recovery time of patients with COVID-19 (Beigel et al., 2020; Wang Y. et al., 2020). As of June 2020, it has been authorized for emergency treatment of COVID-19 in the US, Singapore, Japan, and the UK. CQ was first used to treat malaria, HCQ as its analogue is less toxic than CQ. CQ/HCQ is other drugs under consideration for treating COVID-19. So far, the drugs have been controversial. Some studies have shown that the drugs have significant efficacy in alleviating symptoms (Sarma et al., 2020; Tang et al., 2020), but some studies have reported that CQ/HCQ has potential cardiac side effects, such as prolonging QT interval (Borba et al., 2020). In June 2020, the U.S. Food and Drug Administration revoked the emergency use authorization for HCQ. A clinical trial to evaluate the safety and effectiveness of HCQ for the treatment of COVID-19 has been stopped by the NIH. After its fourth interim analysis, the data and safety monitoring board concluded that while there was no harm, HCQ was unlikely to be beneficial to hospitalized COVID-19 patients (NIH, 2020). In July 2020, WHO discontinued the Solidarity Trial's HCQ and lopinavir/ritonavir arms. Although lopinavir/ritonavir can reduce SARS-CoV-2 viral loads (Lim et al., 2020), the Solidarity Trial's interim results showed that compared with standard treatment, HCQ and lopinavir/ritonavir produce little or no reduction in the mortality of hospitalized COVID-19 patients (WHO, 2020). IFN has been used to treat SARS and MERS, and can improve patient survival (Haagmans et al., 2004; Mustafa et al., 2018); Liu et al. (Liu et al., 2020) reported that the efficacy is not clear for the treatment of COVID-19 using IFN. Hence, a clinical trial has been investigating the efficacy of IFN for the treatment COVID-19 (*Registration number: NCT04254874*). A study (Tian et al., 2020) reported a new coronavirus-specific human monoclonal antibody—CR3022, which can bind SARS-CoV-2 receptor-binding domain, and has potential function to prevent and treat SARS-CoV-2 infections. In addition, there have been some clinical studies investigating the convalescent plasma for the treatment of COVID-19.

A few limitations should be noted in this study. Because COVID-19 is a new disease, its name as well as the name of virus changed many times, so there may be a small number of studies using other names for the registration, which may not have been retrieved. Additionally, due to the worldwide spread of COVID-19, studies will continue to be registered every day and the number of clinical studies is growing, which may also cause some bias. In addition, this study only retrieved trials registered in *ClinicalTrials.gov*. Although *ClinicalTrials.gov* includes more than 3.4 million research studies in 214 countries, some studies may not have been registered on this platform.

In conclusion, the number of registered COVID-19 related clinical studies has increased rapidly since the outbreak, involving epidemiology, risk factors, prevention, diagnosis, treatment, rehabilitation, and psychological aspects. However, some registration parameters are not complete, so it is necessary to strengthen the registration monitoring and supervision for providing high-quality clinical evidence.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding authors.

AUTHOR CONTRIBUTIONS

X-TZ and JJ take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: X-TZ, X-QR, and JJ. Acquisition, analysis, or interpretation of data: All authors. Drafting of the manuscript: L-LM, B-HL, and DH. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: XY, Y-YW, and J-YY. Administrative, technical, or material support: Y-YW, B-HL, and XY. Supervision and review: X-TZ, X-QR, and JJ.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Aslam, A., Imanullah, S., Asim, M., and El-Menyar, A. (2013). Registration of Clinical Trials: Is it Really Needed? *N. Am. J. Med. Sci.* 5 (12), 713–715. doi: 10.4103/1947-2714.123266

Bauchner, H., and Fontanarosa, P. B. (2020). Randomized Clinical Trials and COVID-19: Managing Expectations. *JAMA* 323 (22), 2262–2263. doi: 10.1001/jama.2020.8115

Beigel, J. H., Tomashek, K. M., Dodd, L. E., Mehta, A. K., Zingman, B. S., Kalil, A. C., et al. (2020). Remdesivir for the Treatment of Covid-19 — Preliminary Report. *N. Engl. J. Med.* NEJMoa2007764. doi: 10.1056/NEJMoa2007764

Bhatt, D. L., and Mehta, C. (2016). Adaptive Designs for Clinical Trials. *N. Engl. J. Med.* 375 (1), 65–74. doi: 10.1056/NEJMra1510061

Borba, M. G. S., Val, F. F. A., Sampaio, V. S., Alexandre, M. A. A., Melo, G. C., Brito, M., et al. (2020). Effect of High vs Low Doses of Chloroquine Diphosphate as Adjunctive Therapy for Patients Hospitalized With Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection: A Randomized Clinical Trial. *JAMA Network Open* 3 (4), e208857. doi: 10.1001/jamanetworkopen.2020.8857

Chu, D. K. W., Pan, Y., Cheng, S. M. S., Hui, K. P. Y., Krishnan, P., Liu, Y., et al. (2020). Molecular Diagnosis of a Novel Coronavirus, (2019-nCoV) Causing an Outbreak of Pneumonia. *Clin. Chem.* 66 (4), 549–555. doi: 10.1093/clinchem/hvaa029

COVID-19 Treatment Guidelines Panel (2020). *Coronavirus Disease 2019 (COVID-19) Treatment Guidelines* (National Institutes of Health). Available at: https://www.covid19treatmentguidelines.nih.gov/ (Accessed July 30, 2020).

Esbin, M. N., Whitney, O. N., Chong, S., Maurer, A., Darzacq, X., and Tjian, R. (2020). Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection. *RNA* 26 (7), 771–783. doi: 10.1261/rna.076232.120

Fang, Y., Zhang, H., Xie, J., Lin, M., Ying, L., Pang, P., et al. (2020). Sensitivity of Chest CT for COVID-19: Comparison to RT-PCR. *Radiology* 296 (2), E115–E117. doi: 10.1148/radiol.2020200432

Gautret, P., Lagier, J. C., Parola, P., Hoang, V. T., Meddeb, L., Mailhe, M., et al. (2020). Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int. J. Antimicrob. Agents* 56 (1), 105949. doi: 10.1016/j.ijantimicag.2020.105949

Gheorghe, A., Straehler-Pohl, K., Nkhoma, D., Mughandira, W., Garand, D., Malema, D., et al. (2019). Assessing the feasibility and appropriateness of introducing a national health insurance scheme in Malawi. *Glob. Health Res. Policy* 4, 13. doi: 10.1186/s41256-019-0103-5

Goldacre, B. (2017). The WHO joint statement from funders on trials transparency. *BMJ* 357:j2816. doi: 10.1136/bmj.j2816

Gupta, M., Wahl, B., Adhikari, B., Bar-Zeev, N., Bhandari, S., Coria, A., et al. (2020). The need for COVID-19 research in low- and middle-income countries. *Global Health Res. Policy* 5 (1), 33. doi: 10.1186/s41256-020-00159-y

Haagmans, B. L., Kuiken, T., Martina, B. E., Fouchier, R. A., Rimmelzwaan, G. F., van Amerongen, G., et al. (2004). Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. *Nat. Med.* 10 (3), 290–293. doi: 10.1038/nm1001

Hou, Y. P., Wu, J. L., Tan, C., Chen, Y., Guo, R., and Luo, Y. J. (2019). Sex-based differences in the prevalence of acute mountain sickness: a meta-analysis. *Mil. Med. Res.* 6 (1), 38. doi: 10.1186/s40779-019-0228-3

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395 (10223), 497–506. doi: 10.1016/s0140-6736(20)30183-5

Jin, Y. H., Cai, L., Cheng, Z. S., Cheng, H., Deng, T., Fan, Y. P., et al. (2020). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus, (2019-nCoV) infected pneumonia (standard version). *Mil. Med. Res.* 7 (1):4. doi: 10.1186/s40779-020-0233-6

Johns Hopkins University (2020). *COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU)* (Accessed 2020/7/13 2020).

Li, Z., Yi, Y., Luo, X., Xiong, N., Liu, Y., Li, S., et al. (2020). Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J. Med. Virol.* 92, 1518–1524. doi: 10.1002/jmv.25727

Lim, J., Jeon, S., Shin, H. Y., Kim, M. J., Seong, Y. M., Lee, W. J., et al. (2020). Case of the Index Patient Who Caused Tertiary Transmission of COVID-19 Infection in Korea: the Application of Lopinavir/Ritonavir for the Treatment of COVID-19 Infected Pneumonia Monitored by Quantitative RT-PCR. *J. Korean Med. Sci.* 35 (6), e79. doi: 10.3346/jkms.2020.35.e79

Liu, Y., Yang, Y., Zhang, C., Huang, F., Wang, F., Yuan, J., et al. (2020). Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci. China Life Sci.* 63 (3), 364–374. doi: 10.1007/s11427-020-1643-8

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395 (10224), 565–574. doi: 10.1016/s0140-6736(20)30251-8

Ludvigsson, J. F. (2020). Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. *Acta Paediatr.* 109 (6), 1088–1095. doi: 10.1111/apa.15270

Ma, L. L., Wang, Y. Y., Yang, Z. H., Huang, D., Weng, H., and Zeng, X. T. (2020). Methodological quality (risk of bias) assessment tools for primary and secondary medical studies: what are they and which is better? *Mil. Med. Res.* 7 (1), 7. doi: 10.1186/s40779-020-00238-8

Mehta, H. B., Ehrhardt, S., Moore, T. J., Segal, J. B., and Alexander, G. C. (2020). Characteristics of registered clinical trials assessing treatments for COVID-19: a cross-sectional analysis. *BMJ Open* 10 (6), e039978. doi: 10.1136/bmjopen-2020-039978

Mustafa, S., Balkhy, H., and Gabere, M. N. (2018). Current treatment options and the role of peptides as potential therapeutic components for Middle East Respiratory Syndrome (MERS): A review. *J. Infect. Public Health* 11 (1), 9–17. doi: 10.1016/j.jiph.2017.08.009

NIH (2020). *NIH halts clinical trial of hydroxychloroquine: Study shows treatment does no harm, but provides no benefit* (National Institutes of Health). Available at: https://www.nih.gov/news-events/news-releases/nih-halts-clinical-trial-hydroxychloroquine (Accessed June 20, 2020).

Norris, S. L., Louis, H., Sawin, V. I., Porgo, T. V., Lau, Y. H. A., Wang, Q., et al. (2019). An evaluation of WHO emergency guidelines for Zika virus disease. *J. Evid. Based Med.* 12 (3), 218–224. doi: 10.1111/jebm.12347

RECOVERY Collaborative Group (2020). Dexamethasone in Hospitalized Patients with Covid-19 — Preliminary Report. *N. Engl. J. Med.* NEJMoa2021436. doi: 10.1056/NEJMoa2021436

Ruberg, S. J., and Akacha, M. (2017). Considerations for Evaluating Treatment Effects From Randomized Clinical Trials. *Clin. Pharmacol. Ther.* 102 (6), 917–923. doi: 10.1002/cpt.869

Sarma, P., Kaur, H., Kumar, H., Mahendru, D., Avti, P., Bhattacharyya, A., et al. (2020). Virological and clinical cure in COVID-19 patients treated with hydroxychloroquine: A systematic review and meta-analysis. *J. Med. Virol.* 92 (7), 776–785. doi: 10.1002/jmv.25898

Schulz, K. F., and Grimes, D. A. (2002a). Allocation concealment in randomised trials: defending against deciphering. *Lancet* 359 (9306), 614–618. doi: 10.1016/s0140-6736(02)07750-4

Schulz, K. F., and Grimes, D. A. (2002b). Blinding in randomised trials: hiding who got what. *Lancet* 359 (9307), 696–700. doi: 10.1016/s0140-6736(02)07816-9

Sessler, D. I., and Imrey, P. B. (2015). Clinical Research Methodology 3: Randomized Controlled Trials. *Anesth. Analg.* 121 (4), 1052–1064. doi: 10.1213/ane.0000000000000862

Shang, L., Zhao, J., Hu, Y., Du, R., and Cao, B. (2020). On the use of corticosteroids for 2019-nCoV pneumonia. *Lancet* 395 (10225), 683–684. doi: 10.1016/s0140-6736(20)30361-5

Shirato, K., Nao, N., Katano, H., Takayama, I., Saito, S., Kato, F., et al. (2020). Development of Genetic Diagnostic Methods for Novel Coronavirus 2019 (nCoV-2019) in Japan. *Jpn. J. Infect. Dis.* 73 (4), 304–307. doi: 10.7883/yoken.JJID.2020.061

Tang, W., Cao, Z., Han, M., Wang, Z., Chen, J., Sun, W., et al. (2020). Hydroxychloroquine in patients with mainly mild to moderate coronavirus disease 2019: open label, randomised controlled trial. *BMJ* 369, m1849. doi: 10.1136/bmj.m1849

Tian, X., Li, C., Huang, A., Xia, S., Lu, S., Shi, Z., et al. (2020). Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg. Microbes Infect.* 9 (1), 382–385. doi: 10.1080/22221751.2020.1729069

To, K. K., Tsang, O. T., Chik-Yan Yip, C., Chan, K. H., Wu, T. C., Chan, J. M. C., et al. (2020). Consistent detection of 2019 novel coronavirus in saliva. *Clin. Infect. Dis.* 71 (15), 841–843. doi: 10.1093/cid/ciaa149

Wang, W. G., Hu, H., Song, L., Gong, X. M., Qu, Y. J., and Lu, Z. Y. (2020). Image of pulmonary and diagnosis of atypical novel coronavirus, (2019-nCoV) infected pneumonia: case series of 14 patients. *Yixue Xinzhi Zazhi* 30 (1), 7–9. doi: 10.12173/j.issn.1004-5511.2020.01.04

Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y., et al. (2020). Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 395 (10236), 1569–1578. doi: 10.1016/S0140-6736(20)31022-9

WHO (2020). *WHO discontinues hydroxychloroquine and lopinavir/ritonavir treatment arms for COVID-19* (World Health Organization). Available at: https://www.who.int/news-room/detail/04-07-2020-who-discontinues-hydroxychloroquine-and-lopinavir-ritonavir-treatment-arms-for-covid-19 (Accessed July 4, 2020).

Yu, F., Du, L., Ojcius, D. M., Pan, C., and Jiang, S. (2020). Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China. *Microbes Infect.* 22 (2), 74–79. doi: 10.1016/j.micinf.2020.01.003

Zhang, J. J., Dong, X., Cao, Y. Y., Yuan, Y. D., Yang, Y. B., Yan, Y. Q., et al. (2020). Clinical characteristics of 140 patients infected by SARS-CoV-2 in Wuhan, China. *Allergy* 75 (7), 1730–1741. doi: 10.1111/all.14238

Zhang, R., Li, Y., Zhang, A. L., Wang, Y., and Molina, M. J. (2020). Identifying airborne transmission as the dominant route for the spread of COVID-19. *Proc. Natl. Acad. Sci.* 117 (26), 14857–14863. doi: 10.1073/pnas.2009637117

Zhou, S. S., Lukula, S., Chiossone, C., Nims, R. W., Suchmann, D. B., and Ijaz, M. K. (2018). Assessment of a respiratory face mask for capturing air pollutants and pathogens including human influenza and rhinoviruses. *J. Thorac. Dis.* 10 (3), 2059–2069. doi: 10.21037/jtd.2018.03.103

Zhou, D., Dai, S.-M., and Tong, Q. (2020). COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. *J. Antimicrob. Chemother.* 75 (7), 1667–1670. doi: 10.1093/jac/dkaa114

Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579 (7798), 270–273. doi: 10.1038/s41586-020-2012-7

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Positive RT-PCR Test Results in 420 Patients Recovered From COVID-19 in Wuhan: An Observational Study

Shaobin He[1,2†], Jiaxing Tian[3†], Xiaodong Li[2,4†], Yana Zhou[5†], Mingzhong Xiao[4], Ying Zhang[6], Xiaojun Min[7], Xiuyang Li[8], De Jin[3], Qing Zhang[9], Yujiao Zheng[3,10], Jia Ke[11], Qingwei Li[3], Junxiu Tao[4], Ping Song[12], Han Wang[3], Yi Lv[4], Qiyou Ding[3,10], Shuang Tang[3,10], Jiaran Lin[3,10], Zhaoyuan Jiang[12], Zijun Zhang[12], Juexian Song[13], Fengmei Lian[3] and Xiaolin Tong[3*]*

[1] Hubei Hospital of Traditional Chinese Medicine, Wuhan, China, [2] Hubei University of Chinese Medicine, Wuhan, Wuhan, China, [3] Departments of Endocrinology, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [4] Liver Disease Branch, Hubei Hospital of Traditional Chinese Medicine, Wuhan, China, [5] Department of Oncology, Hubei Hospital of Traditional Chinese Medicine, Wuhan, China, [6] Center for Evidence-based Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China, [7] Department of Endocrinology, Hubei Hospital of Traditional Chinese Medicine, Wuhan, China, [8] Medical Affairs Department, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [9] Department of Orthopedics, Hubei Hospital of Traditional Chinese Medicine, Wuhan, China, [10] Graduate School, Beijing University of Chinese Medicine, Beijing, China, [11] Department of Pulmonary Medicine, Hubei Hospital of Traditional Chinese Medicine, Wuhan, China, [12] International Cooperation Department, China Academy of Chinese Medical Sciences, Beijing, China, [13] Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China

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Edited by:
Heike Wulff,
University of California, Davis,
United States

Reviewed by:
Gaurav Deshpande,
Bristol Myers Squibb, United States
Brian Godman,
Karolinska Institutet (KI), Sweden

***Correspondence:**
Xiaolin Tong
tongxiaolin@vip.163.com
Fengmei Lian
lfm565@sohu.com

[†]These authors have contributed equally to this work

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Objective: During the follow-up of patients recovered from coronavirus disease 2019 (COVID-19) in the quarantine and observation period, some of the cured patients showed positive results again. The recurrent positive RT-PCR test results drew widespread concern. We observed a certain number of cured COVID-19 patients with positive RT-PCR test results and try to analyze the factors that caused the phenomenon.

Methods: We conducted an observational study in COVID-19 patients discharged from 6 rehabilitation stations in Wuhan, China. All observed subjects met the criteria for hospital discharge and were in quarantine. Data regarding age, sex, body mass index (BMI), course of disease, comorbidity, smoking status and alcohol consumption, symptoms in and out of quarantine, and intervention were collected from the subjects' medical records and descriptively analyzed. The main outcome of this study was the RT-PCR test result of the observed subjects at the end of quarantine (negative or positive). Logistic regression analysis was used to identify the influencing factors related to recurrent positive RT-PCR test results.

Results: In this observational study, 420 observed subjects recovered from COVID-19 were included. The median age was 56 years, 63.6% of the subjects were above 50 years old, and 50.7% (213/420) were female. The most common comorbidities were hypertension [26.4% (111/420)], hyperlipidemia [10.7% (45/420)], and diabetes [10.5% (44/420)]. 54.8% (230/420) manifested one or more symptoms at the beginning of the observation period, the most common symptoms were cough [27.6% (116/420)], shortness of breath 23.8% (100/420)], and fatigue [16.2% (68/420)], with fever rare [2.6% (11/420)]. A total of 325 subjects were exposed to comprehensive intervention; 95

subjects were absence of intervention. The recurrence rate of positive RT-PCR test results with comprehensive intervention was 2.8% (9/325), and that with no intervention was 15.8% (15/95). The results of logistic regression analysis showed that after adjusted for factors such as age, sex, and comorbidity and found out that comprehensive intervention was correlated with the recurrent positive RT-PCR test results. There was appreciably less recurrence in the comprehensive intervention group.

Conclusions: The factors related to positive RT-PCR test results in observed subjects recovered from COVID-19 were age, comorbidity, and comprehensive intervention, among which comprehensive intervention might be a protective factor.

Clinical Trial Registration: Chictr.org.cn, identifier ChiCTR2000030747.

Keywords: coronavirus disease 2019, comprehensive intervention, correlation factor analysis, recurrence rate, positive RT-PCR test result

INTRODUCTION

By March 11th, 2020, 121,133 cases were diagnosed as coronavirus disease 2019 (COVID-19) globally. In China, 80,967 cases have been diagnosed, among which 61661 have been cured and discharged from the hospital (Chinese Center for Disease Control and Prevention). In view of sequelae in cured patients with severe acute respiratory syndrome (SARS), numerous discharged patients have drawn public attention. Recently, it was reported that some COVID-19 patients who had met the criteria for hospital discharge (absence of clinical symptoms and radiological abnormalities with 2 consecutive negative RT-PCR test results) showed positive RT-PCR test results for COVID-19 nucleic acid later (Lan et al., 2020). The patients usually had no or mild clinical symptoms; however, their health status and infectivity were unclear, which caused widespread concern to the key points which affected the control of the disease, including the complexity of COVID-19, discharge criteria, reinfection after discharge, infectivity of discharged patients with positive RT-PCR test results, quality of nucleic acid kit and specimen sampling, and obstructed to epidemic prevention and control. Currently, most researchers focus on the epidemiological characteristics of COVID-19 patients, as well as the clinical manifestations and efficacy outcomes. However, few studies have been conducted on patients who have recovered and been discharged, which has significantly affected our complete understanding of the disease. In Wuhan, with the implementation of 14-day quarantine measures for discharged COVID-19 patients, we observed a certain number of cured COVID-19 patients with RT-PCR test results in and out of the quarantine and tried to analyze the factors that caused this phenomenon. The study was approved by the Medical Ethics Committee of Hubei Provincial Hospital of Traditional Chinese Medicine (no. HBZY2020-C01-01).

METHODS

Study Design and Participants

We conducted an observational study using data from six rehabilitation stations: Wuhan Vocational College of Software and Engineering (WVCSE) rehabilitation station, the City Economic Hotel on Chunghwa Road, Galaxy Kindom Hotel on Yangyuan Street, Lavande Hotel on Jiajiashan Street, You Melody Hotel on Liangdao Street, and Home Inn on Liangdao Street. All the COVID-19 patients observed in this study had been hospitalized and discharged before, so they were all tested negative for RT-PCR when included. The current COVID-19 discharge criteria are as follows: 1) body temperature is back to normal for more than 3 days; 2) respiratory symptoms improve obviously; 3) pulmonary imaging shows obvious absorption of inflammation, 4) nucleic acid tests negative twice consecutively on respiratory tract samples such as sputum and nasopharyngeal swabs (sampling interval being at least 24 h). All observed subjects met the above discharge criteria and were in quarantine. If the RT-PCR test was still negative after 14 days from discharge, then they can be released from quarantine. During the observed period, some patients were administered comprehensive intervention, and some were not. The comprehensive interventions included: (1) Baduanjin exercise (Zhao et al., 2019), was taught by a professional instructor combined with recorded videos. The exercise time was 15 min per day during 10:00–10:15 in the morning or 15:00–15:15 in the afternoon. (2) Foot baths (Vyas et al., 2019) were performed 1 h before bedtime for 20 min daily. The temperature of water in the foot bath should be controlled at 38–40°C, and people with skin ulcers on their feet should not undergo this therapy. (3) Moxibustion with acupoint application (Shou et al., 2020), which was a Type II acupoint plaster for intervening cough from Wuhan Guojiu Technology Development Co., Ltd. (Registration no. Hubei Drug Administration Machinery (Zhun) Zi 2002 no. 2260633); the selected acupoints included CV22 and GV14. The instructions were to apply 1 paste of the Type II Acupoint plaster for intervening cough on acupoints CV22 and GV14, once a day for 12 h. Pregnant women and patients with diabetes, skin allergies, skin ulceration, and acute contusion bleeding disorders were prohibited from using this therapy. (4) Tongzhi Granule, administered to 1 bag (dissolved in 200 mL of water at 95°C) per day, 30 min after breakfast, and 30 min after dinner. (5) Wuhan Kangyi Decoction, administered

to 2 bags (dissolved in 200 mL of water at 95°C) 30 min after breakfast and dinner, respectively. The above therapies can be chosen and combined based on individual symptoms. Considering that we need to evaluate the recurrent rate of positive RT-PCR test results of the population, we excluded the suspected cases and clinically diagnosed cases of COVID-19; convalescents in quarantine with RT-PCR testing were included. In the study, subjects were divided into the comprehensive intervention and the no intervention.

Procedures

The results of this study were analyzed and reported in accordance with the STROBE guidelines. Prior to January 23, 2020, laboratory confirmation of SARS-CoV-2 was performed at the Chinese Center for Disease Control and Prevention (CDC); subsequently, laboratory confirmation was performed at certified tertiary hospitals. The RT-PCR test was based on the criteria provided by the World Health Organization (WHO) (World Health Organization, 2020). We obtained medical records of diagnosed COVID-19 patients who were discharged from the hospital and were in quarantine from February 22, 2020 to March 10, 2020 (National Health Commission of the People's Republic of China, 2020a). The nucleic acid kit (fluorescent RT-PCR) was recommended by the CDC, and extraction of nucleic acid from clinical samples (including uninfected cultures that served as negative controls) was performed as the description of the manufacturer (BGI Biotechnology Co., Ltd). Data on demographic and clinical characteristics, comorbidity, course of disease, smoking status, and alcohol consumption were extracted. Symptoms of the observed subjects in and out of the rehabilitation station and comprehensive intervention were also recorded. If the relevant information was missing, we directly contacted the patient's family. Data for the study were collected and examined manually by two researchers, and differences were resolved through consultation by a third researcher.

Outcomes

The primary outcome of this study was the RT-PCR test result of the observed subjects at the end of quarantine (negative or positive).

In addition, we performed a descriptive analysis of the demographic characteristics. Age, sex, body mass index (BMI), course of disease, comorbidity, smoking status and alcohol consumption, symptoms in and out of quarantine, and intervention were descriptively analyzed. We also compared the patients according to different interventions, and each intervention was considered a factor. Logistic regression analysis was used to compare positive RT-PCR test results in convalescents with intervention, age, sex, BMI, course of disease, comorbidity, smoking status, and alcohol consumption and to identify the influencing factors related to recurrent positive RT-PCR test results.

Statistical Analysis

Numerical variables were summarized as mean (± SD) if the data are normally distributed or median variables were presented (interquartile range, IQR) if they are not. The data of the categorical variables were described as counts and percentages. The characteristics of the subjects and the different interventions (comprehensive invention and no intervention) were described. The characteristic variables included age, sex, BMI, comorbidity, course of disease, smoking status, alcohol consumption, and symptoms in and out of quarantine. Univariate analysis was used to analyze the characteristics of different interventions. Multivariable logistic regression was used to analyze possible independent factors that influence recurrent positive RT-PCR test results. P-values < 0.05 were considered statistically significant. The OR value and 95%CI were used to estimate the effect size. Statistical analyses were performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, United States).

RESULTS

By March 10, 2020, the data of 607 cases from 6 rehabilitation stations in Wuhan was collected, including 84 suspected cases, 29 clinically diagnosed cases and 494 former diagnosed cases. 420 former diagnosed patients have completed the RT-PCR testing and were included in the study, among which 325 observed subjects were administered comprehensive intervention, 95 subjects didn't receive any intervention (**Figure 1**).

Demographic and Clinical Characteristics

In 420 observed subjects, the median age was 56 years, 63.6% of the subjects were above 50 years old, 50.7% (213/420; 95% CI: 45.9–55.5%) were female. 52.2% (219/420; 95% CI:47.4–56.9%) of the subjects were overweight or obese (BMI≥24), and 41.0% (172/420; 95% CI: 36.2–45.7%) had one or more comorbidities. The most common comorbidities were hypertension [26.4% (111/420; 95% CI: 22.2–30.6%)], hyperlipidemia [10.7% (45/420; 95% CI:7.8–13.7%)] and diabetes [10.5% (44/420; 95% CI: 7.5–13.4%)]. 54.8% (230/420; 95% CI: 50–59.5%) manifested one or more symptoms at the beginning of the observation period, the most common symptoms were cough [27.6% (116/420; 95% CI: 23.3–31.9%)], shortness of breath 23.8% (100/420; 95% CI: 19.7–27.9%)] and fatigue [16.2% (68/420; 95% CI:12.7–19.7%)], with fever rare [2.6% (11/420; 95% CI:1.1–4.1%)]. 10.5% (44/420; 95% CI:7.5–13.4%) of the subjects were smokers and 10.5% (44/420; 95% CI: 7.5–13.4%) with alcohol consumption. The duration of disease was defined as time from onset to the time of RT-PCR testing. The subjects' median course of disease was 40 days.

Between 325 subjects with comprehensive intervention and 95 subjects without intervention, there were differences in the age (54 vs. 58), comorbidity (36.6 vs. 55.8%), and symptom (51.4 vs. 66.3%) at the beginning of the observation period. Subjects in the non-intervention group had more comorbidities and symptoms at the beginning of the observation period and were older. While there was no significant difference between the two groups in terms of sex, BMI, course of disease, smoking status, and alcohol consumption (**Table 1**).

Application of Comprehensive Intervention

Comprehensive intervention included Baduanjin exercise, Chinese herbal medicine, moxibustion with acupoint

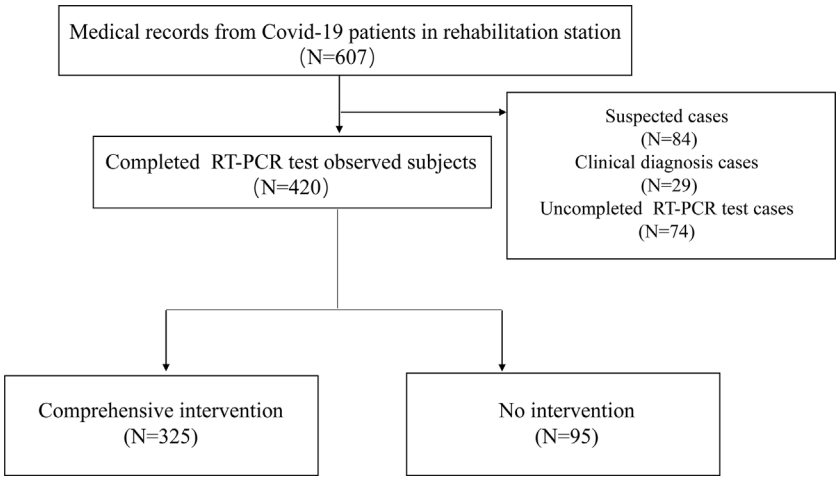

FIGURE 1 | Flow chart of the observational research.

application, and foot baths. Baduanjin exercise (100%) and Chinese herbal medicine prescriptions (90.5%) were most widely used (**Table 2**). The frequency of patients receiving various treatment combinations in the intervention group was shown in the supplementary materials (**Table S1**).

Symptoms Before RT-PCR Testing

57.4% (241/420; 95% CI: 52.7–62.1%) subjects still manifested symptoms at the end of the observation period, 22.6% (95/420; 95% CI: 18.6–26.6%) of them showed insomnia, other common symptoms included cough [22.4%(94/420; 95% CI: 18.4–26.4%)], shortness of breath [21.0% (88/420; 95% CI: 17.1–24.8%)] and sweating [16.4% (69/420; 95% CI: 12.9–20%)] (**Table 1**). Besides, there was a difference between no intervention and comprehensive intervention in proportion of subjects accompanied symptoms (53.2 vs. 71.6%).

RT-PCR Test Results

At the end of the observation period, 420 subjects had completed at least one RT-PCR test. It was found that the overall recurrent rate of positive RT-PCR test results was 5.7% (24/420; 95% CI: 3.5–7.9%), 2.8% (9/325; 95% CI: 1.0–4.6%) in the comprehensive intervention group, and 15.8% (15/95; 8.5–23.1%) in the non-intervention group (**Table 2**).

Analysis of Positive RT-PCR Test Results

We performed a logistic regression analysis using factors including comprehensive intervention (yes/no), age, sex, BMI, course of disease, symptom, comorbidity, smoking status, and alcohol consumption to analyze the relations of positive RT-PCR test results. The results of univariate analysis showed that age, comorbidity, and intervention of the observed subjects were related to positive RT-PCR test results ($P < 0.05$). Multivariate analysis revealed that intervention was related to positive RT-PCR test results ($P < 0.05$), suggesting that comprehensive intervention method might be a protective factor for positive RT-PCR test results (**Table 3**). Detailed logistic regression analysis was shown in the supplementary materials (**Tables S2**-**S4**).

DISCUSSION

On February 25, 2020, the Guangdong Provincial CDC released preliminary statistics, which showed that the proportion of cured patients presenting with positive RT-PCR test results discharged in Guangdong Province was approximately 14%. Another study reported that after four COVID-19 patients (all medical personnel) were cured, RT-PCR test result from pharyngeal swabs appeared positive again (Lan et al., 2020). The Eighth People's Hospital of Guangzhou City also continued to follow up COVID-19 patients discharged from hospital, finding that the recurrent rate of positive RT-PCR test results was 9.6%. Repeated fluctuation-positive RT-PCR test results have drawn widespread attention globally. It's unclear whether patients who meet the current clinical recovery criteria are completely cured. In the process of continuously exploring the disease, we have focused on whether symptoms recurred in the population, the time when the RT-PCR test result turned negative again, the infectivity of the population, the reason, significance, and related factors of positive RT-PCR results in cured COVID-19 patients.

The causes of positive RT-PCR test results in cured patients have been researched at present (Ling et al., 2020). Considering the biological characteristics of SARS-CoV-2 and reduced reinfection of cured patients, the positive RT-PCR test results are probably due to the presence of virus residues in the body. Moreover, negative RT-PCR test results cannot rule out the possibility of COVID-19 (National Health Commission of the People's Republic of China, 2020b). There are some factors that may cause false negatives, which could lead to misjudgment of

TABLE 1 | Characteristics of study observed person.

Characteristic	All patients	Comprehensive intervention	No intervention
Age			
Median (IQR)-year	56(43–63.75)	54(42–62)	58(48–68)
Distribution-no./total no. (%; 95% CI)			
0–14 years	1/420(0.2; -0.2–0.7)	0/325(0)	1/95(1.1; -1.0–3.1)
15–49 years	152/420(36.2;31.6–40.8)	126/325(38.8;33.5–44.1)	26/95(27.4; 18.4–36.3)
50–64 years	172/420(41.0;36.2–45.7)	139/325(42.8;37.4–48.1)	33/95(34.7; 25.2–44.3)
≥65 yeats	95/420(22.6;18.6–26.6)	60/325(18.5;14.2–22.7)	35/95(36.8; 27.1–46.5)
Sex			
Female sex-no./total no. (%)	213/420(50.7;45.9–55.5)	160/325(49.2;43.8–54.7)	53/95(55.8; 45.8–65.8)
Body mass index			
Median (IQR)-kg/m^2	24.05(21.99–26.02)	24.06(21.97–26.18)	24.03(22.49–25.48)
Distribution-no./total no. (%; 95% CI)			
<18.5kg/m^2	12/420(2.9;1.3–4.5)	10/325(3.1;1.2–0.5)	2/95(2.1; -0.8–5)
18.5 ≤ BMI<24kg/m^2	189/420(45.0;40.2–49.8)	145/325(44.6;39.2–50)	44/95(46.3; 36.3–56.3)
24≤BMI<27kg/m^2	146/420(34.8;30.2–39.3)	111/325(34.2;29–39.3)	35/95(36.8; 27.1–46.5)
BMI≥27kg/m^2	73/420(17.4;13.8–21)	59/325(18.2;14–22.3)	14/95(14.7; 7.6–21.9)
Course of disease			
Median (IQR)-d	40(33–43)	39(33–43)	40(35–44)
Symptoms during observation period -no./total no. (%; 95% CI)			
Total	230/420(54.8; 50–59.5)	167/325(51.4; 46.0–56.8)	63/95(66.3; 56.8–75.8)
Cough	116/420(27.6; 23.3–31.9)	82/325(25.2; 20.5–30.0)	34/95(35.8; 21.6–45.4)
Shortness of breath	100/420(23.8; 19.7–27.9)	75/325(23.1; 18.5–27.7)	25/95(26.3; 17.5–35.2)
Fatigue	68/420(16.2; 12.7–19.7)	54/325(16.6; 12.6–20.7)	14/95(14.7; 7.6–21.9)
Insomnia	37/420(8.8; 6.1–11.5)	8/325(2.5; 0.8–4.1)	29/95(30.5; 21.3–39.8)
Inappetence	36/420(8.6; 5.9–11.2)	26/325(8.0; 5.1–10.9)	10/95(10.5; 4.4–16.7)
Sweating	30/420(7.1; 4.7–9.6)	12/325(3.7; 1.6–5.7)	18/95(18.9; 11.1–26.8)
Diarrhea	29/420(6.9; 4.5–9.3)	22/325(6.8; 4–9.5)	7/95(7.4; 2.1–12.6)
Limb pain	22/420(5.2; 3.1–7.4)	22/325(6.8; 4–9.5)	–
Thirsty	18/420(4.3; 2.3–6.2)	1/325(0.3; -0.3–0.9)	17/95(17.9; 10.2–25.6)
Nausea and vomiting	15/420(3.6; 1.8–5.3)	15/325(4.6; 2.3–6.9)	–
Fever	11/420(2.6; 1.1–4.1)	11/325(3.4; 1.4–5.4)	0/95(0)
Constipation	9/420(2.1; 0.8–3.5)	–	9/95(9.5; 3.6–15.4)
Fear of wind	3/420(0.7; -0.1–1.5)	2/325(0.6; -0.2–1.5)	1/95(1.1; -1.0–3.1)
Symptoms after observation period -no./total no. (%; 95% CI)			
Total	241/420(57.4; 52.7–62.1)	173/325(53.2; 47.8–58.7)	68/95(71.6; 62.5–80.6)
Insomnia	95/420(22.6; 18.6–26.6)	63/325(19.4; 15.1–23.7)	32/95(33.7; 24.2–43.2)
Cough	94/420(22.4; 18.4–26.4)	63/325(19.4; 15.1–23.7)	31/95(32.6; 23.2–42.1)
Shortness of breath	88/420(21.0; 17.1–24.8)	55/325(16.9; 12.8–21.0)	33/95(34.7; 25.2–44.3)
Sweating	69/420(16.4; 12.9–20)	53/325(16.3; 12.3–20.3)	16/95(16.8; 9.3–24.4)
Expectoration	55/420(13.1; 9.9–16.3)	39/325(12.0; 8.5–15.5)	16/95(16.8; 9.3–24.4)
Thirsty	52/420(12.4; 9.2–15.5)	29/325(8.9; 5.8–12)	23/95(24.2; 15.6–32.8)
Fatigue	38/420(9.0; 6.3–11.8)	17/325(5.2; 2.8–7.7)	21/95(22.1; 13.8–30.4)
Diarrhea	26/420(6.2; 3.9–8.5)	16/325(4.9; 2.6–7.3)	10/95(10.5; 4.4–16.7)
Inappetence	18/420(4.3; 2.3–6.2)	10/325(3.1; 1.2–5.0)	8/95(8.4; 2.8–14.0)
Limb pain	17/420(4.0; 2.2–5.9)	17/325(5.2; 2.8–7.7)	–
Constipation	16/420(3.8; 2.0–5.6)	5/325(1.5; 0.2–2.9)	11/95(11.6; 5.1–18)
Fever	9/420(2.1; 0.8–3.5)	3/325(0.9; -0.1–2.0)	6/95(6.3; 1.4–11.2)
Comorbidity			
Total	172/420(41.0; 36.2–45.7)	119/325(36.6; 31.4–41.9)	53/95(55.8; 45.8–65.8)
Hypertension	111/420(26.4; 22.2–30.6)	87/325(26.8; 22–31.6)	24/95(25.3; 16.5–34)
Hyperlipidemia	45/420(10.7; 7.8–13.7)	35/325(10.8; 7.4–14.1)	10/95(10.5; 4.4–16.7)
Diabetes	44/420(10.5; 7.5–13.4)	31/325(9.5; 6.3–12.7)	13/95(13.7; 6.8–20.6)
Coronary heart disease	23/420(5.5; 3.3–7.7)	16/325(4.9; 2.6–7.3)	7/95(7.4; 2.1–12.6)
Hepatopathy	13/420(3.1; 1.4–4.8)	3/325(0.9; -0.1–2.0)	10/95(10.5; 4.4–16.7)
Chronic bronchitis	12/420(2.9; 1.3–4.5)	1/325(0.3; -0.3–0.9)	11/95(11.6; 5.1–18)
Hyperuricemia	7/420(1.7; 0.4–2.9)	7/325(2.2; 0.6–3.7)	–
Malignant tumor	6/420(1.4; 0.3–2.6)	3/325(0.9; -0.1–2.0)	3/95(3.2; -0.4–6.7)
Chronic nephritis	4/420(1.0; 0.0–1.9)	1/325(0.3; -0.3–0.9)	3/95(3.2; -0.4–6.7)
Cerebral apoplexy	4/420(1.0; 0.0–1.9)	1/325(0.3; -0.3–0.9)	3/95(3.2; 0.4–6.7)
Smoking status-no./total no. (%; 95% CI)			
Yes	44/420(10.5; 7.5–13.4)	35/325(10.8; 7.4–14.1)	9/95(9.5; 3.6–15.4)
No	376/420(89.5; 86.6–92.5)	290/325(89.2; 85.9–92.6)	86/95(90.5; 84.6–96.4)
Alcohol consumption-no./total no. (%; 95% CI)			
Yes	44/420(10.5; 7.5–13.4)	32/325(9.8; 6.6–13.1)	12/95(12.6; 6.0–19.3)
No	376/420(89.5; 86.6–92.5)	293/325(90.2; 86.9–93.4)	83/95(87.4)

TABLE 2 | Comprehensive intervention and Positive RT-PCR test.

	All patients	Comprehensive intervention	No intervention
Comprehensive intervention-no./total no. (%; 95% CI)			
Baduanjin exercise		325/325(100)	
Tongzhi granule	–	294/325(90.5; 87.3–93.7)	–
Wuhan Kangyi decoction	–	39/325(12.0; 8.5–15.5)	–
Moxibustion with acupoint application	–	90/325(27.7; 22.8–32.6)	–
Foot bath	–	19/325(5.8; 3.3–8.4)	–
Positive RT-PCR test-no./total no.(%; 95% CI)	24/420(5.7; 3.5–7.9)	9/325(2.8; 1.0–4.6)	15/95(15.8; 8.5–23.1)

TABLE 3 | Univariate Analysis and Multivariate Analyses for the Positive RT-PCR test.

	Univariate Analyses		Multivariate analyses	
	P	OR (95% CI)	P	OR (95% CI)
Management (comprehensive intervention vs. no intervention)	<0.001	0.152 (0.064, 0.360)	<0.001[1]	0.169 (0.070, 0.408)
			<0.001[2]	0.169 (0.070, 0.408)
			<0.001[3]	0.162 (0.066, 0.395)
			<0.001[4]	0.169 (0.069, 0.412)
			<0.001[5]	0.166 (0.067, 0.407)
			<0.001[6]	0.165 (0.067, 0.406)
Sex (M vs. F)	0.623	1.231 (0.538, 2.813)	0.448	1.394 (0.592, 3.286)
			0.451	1.395 (0.587, 3.318)
			0.170	1.891 (0.760, 4.705)
			0.198	1.825 (0.730, 4.564)
			0.207	1.806 (0.721, 4.525)
			0.210	1.800 (0.718, 4.510)
Age	0.026	1.040 (1.005, 1.076)	0.127	1.026 (0.993, 1.062)
			0.129	1.026 (0.992, 1.062)
			0.093	1.030 (0.995, 1.065)
			0.189	1.024 (0.988, 1.062)
			0.184	1.025 (0.989, 1.062)
			0.183	1.025 (0.989, 1.062)
BMI	0.999	1.000 (0.890, 1.123)	0.988	0.999 (0.893, 1.118)
			0.913	0.994 (0.888, 1.112)
			0.755	0.981 (0.871, 1.105)
			0.716	0.978 (0.869, 1.102)
			0.704	0.977 (0.868, 1.101)
Smoking Status (Y vs N)	0.998	NA*	0.997	NA
			0.997	NA
			0.997	NA
			0.997	NA
Alcohol Use (Y vs. N)	0.725	0.766 (0.174, 3.374)	0.951	0.951 (0.188, 4.804)
			0.998	0.998 (0.197, 5.051)
			0.958	1.045 (0.206, 5.309)
			0.946	1.058 (0.208, 5.373)

**Estimators were not available because one cell contained zero value.*
[1]Model included site, age and sex.
[2]Model included site, age, sex and BMI.
[3]Model included site, age, sex, BMI, smoking status and alcohol use.
[4]Model included site, age, sex, BMI, smoking status, alcohol use and disease history.
[5]Model included site, age, sex, BMI, smoking status, alcohol use, disease history and symptoms status when entering isolation site.
[6]Model included site, age, sex, BMI, smoking status, alcohol use, disease history, symptoms status when entering isolation site, and the duration from initial symptoms onset to nucleic retest.

cure in patients. These potential factors include sensitivity of the RNA extraction kit and specimen collection process methods. The production of kits has faced increased demands due to a sudden epidemic situation, the research and development time is extremely limited, the process is simplified, and the quality of the kits is unstable, all of which affect the sensitivity of the kits. Specimen collection method, collection time, storage and transportation also have a certain impact on the RT-PCR test result (Yang et al., 2020). For example, when sampling with swabs, if the sampling time is too short to collect the virus RNA, false negative results will occur. In addition, the application of medicine is also an important factor in the misjudgment of negative RT-PCR test results. The use of glucocorticoid and other medicines were found to negatively affect the body's immune balance and inhibit inflammatory response, causing delays in eliminating the virus (Torres et al., 2015). According to the latest research, SARS-CoV-2 can detoxify for up to 37 days (Zhou et al., 2020). Viral pneumonia usually maintains a longer recovery period, including COVID-19. This characteristic manifestation of COVID-19 has increased the difficulty of objectively and precisely assessing the patient's lung recovery using computed tomography (CT), and therefore affects accurate recovery judgment. In addition, recurrent positive RT-PCR test results are also associated with the patients' autoimmune function system and comorbidity (Ling et al., 2020).

In response to the increasing number of reports of positive RT-PCR test results in recovered COVID-19 patients, the Center for Disease Prevention and Control has made an immediate response and adjustment, which is to strengthen the continuous investigation and detection of RT-PCR test during the quarantine of cured patients, and strengthen follow-up and health guidance. Experts suggest that if recurrent positive RT-PCR test results occur, a quick re-test in the short term will benefit to rule out the misjudgment caused by technology. RT-PCR tests of nasopharyngeal swabs combined with anal swabs also contribute to improving the accuracy of the assessment of viral status. In addition, scientists have proposed a layered discharge strategy for different types of patients, which reflects more individualized assessment methods consistent with clinical practice, by increasing the number of RT-PCR tests and the criteria for patients' hospital discharge and quarantine to reduce positive conversion ratio. It is worth noting that antibody testing has been incorporated into the diagnostic standards of COVID-19, and new corresponding measures, including viral antibody testing, have gradually begun to introduced in discharged patients to promote objective assessment of patients' recovery status.

In the course of clinical practice, we found that patients who received comprehensive intervention had fewer events of positive RT-PCR test results recurrence than patients who did not receive intervention. In this study, we explored the factors that influence the RT-PCR test results. By univariate logistic regression analysis, it was found that age, combined underlying diseases,

and intervention methods were correlated with positive RT-PCR test results recurrence ($P < 0.05$). Previously published literature has reported that age, gender, and underlying diseases were risk factors for COVID-19 (Li et al., 2020; Wang et al., 2020; Zhou et al., 2020). Considering the potential influence of age, gender and underlying diseases on the nucleic acid reactivation results, which may interfere with the actual relationship between the intervention methods and PT-PCR results. Consequently, factors such as age, gender, and underlying diseases need to be corrected. After adjusting for these factors in multivariate analysis, we found the actual relationship between the intervention methods and positive RT-PCR test results recurrence. That was, the comprehensive intervention mode is the protective factors of positive RT-PCR test results recurrence. The comprehensive interventions in our study including Baduanjin exercise, foot baths, moxibustion with acupoint application and Chinese medicine may strengthen the immune system (Tong et al.; Zou et al., 2018), restore the body's metabolic balance, and promote elimination of residual viruses from the body; all these effects might reduce the proportion of positive RT-PCR test results in discharged COVID-19 patients.

Though positive RT-PCR test results and the ability to transmit the virus in patients who have been discharged, still remain unreasonable explanation. The comprehensive intervention therapy used in our study can reduce the occurrence of positive RT-PCR test, and its mechanism may be related to improving the body's immune function, promoting the recovery of the body's metabolic balance, and accelerating the excretion of residual viruses in the body. The comprehensive intervention therapy could be recommended for COVID-19 patients who have been discharged from hospital, and entered the rehabilitation stations for the 14 days period of clinical observation. The comprehensive intervention therapy can promote early recovery of patients, reduce the recurrence of positive RT-PCR test results and prevent secondary transmission, which provides a reference experience for the prevention and control of the recurrence phenomenon.

However, the specific targets and mechanisms of the intervention need to be further explored. Considering the small sample size of our study and the fact that the method of quantitative detection of viral antibody has not been adopted, the results of this study need further confirmation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Hubei Provincial Hospital of Traditional Chinese Medicine (no. HBZY2020-C01-01). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XT, FL, SH, XDL, and YiZ designed the study, took responsibility for the integrity of the data and the accuracy of the data analysis, and had final responsibility for the decision to submit for publication. MX, XM, XYL, QZ, JK, JuT, and YL contributed to data acquisition. YaZ analyzed the data. FL and YaZ contributed to data interpretation. YaZ, QL, JuT, FL, and YaZ contributed to data sorting and cleaning. FL, JiT, DJ, YuZ, QL, QD, ST, JL, PS, HW, ZJ, ZZ, and JS drafted the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.549117/full#supplementary-material

REFERENCES

Chinese Center for Disease Control and Prevention *Global distribution of coronavirus disease 2019 (COVID-19) cases*. Available at: http://2019ncov.chinacdc.cn/2019-nCoV/global.html (Accessed March 11, 2020).

Lan, L., Xu, D., Ye, G., Xia, C., Wang, S., Li, Y., et al. (2020). Positive RT-PCR Test Results in Patients Recovered From COVID-19. *JAMA* 323, 1502–1503. doi: 10.1001/jama.2020.2783

Li, X., Xu, S., Yu, M., Wang, K., Tao, Y., Zhou, Y., et al. (2020). Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J. Allergy Clin. Immunol.* S0091-6749 (20), 30495–30494. doi: 10.1016/j.jaci.2020.04.006

Ling, Z., Kui, L., and Huiguo, L. (2020). Cause analysis and treatment strategies of recurrent positive nucleic acid test with Coronavirus disease 2019 (COVID-19) patients after discharge from hospital. *Chin. J. Tuberc. Respir. Dis.* 43, E028–E028.

National Health Commission of the People's Republic of China. (2020a). *Diagnosis and Treatment Guideline of coronavirus disease 2019 (7th)*. Available at: http://www.nhc.gov.cn/yzygj/s7652m/202003/a31191442e29474b98bfed5579d5af95.shtml.

National Health Commission of the People's Republic of China. (2020b). *Laboratory testing technical guideline for Coronavirus disease 2019 (COVID-19)*. Available at: http://www.nhc.gov.cn/yzygj/s7659/202001/b91fdab7c304431eb082d67847d27e14.shtml [Accessed March 4, 2020].

Shou, Y., Hu, L., Zhang, C., Xu, S., Jin, Q., Huang, L., et al. (2020). Efficacy of acupuncture at three nasal acupoints plus acupoint application for perennial allergic rhinitis: A multicenter, randomized controlled trial protocol. *Trials* 21, 110. doi: 10.1186/s13063-019-4039-3

Tong, X., Li, X., Zhao, L., Li, Q., Yang, Y., Lin, Y., et al. (2020). To discuss the TCM prevention and treatment strategies of Coronavirus disease 2019 from the perspective of Cold-dampness plague. *J. Tradit. Chin. Med.* 61, 465–553.

Torres, A., Sibila, O., Ferrer, M., Polverino, E., Menendez, R., Mensa, J., et al. (2015). Effect of Corticosteroids on Treatment Failure Among Hospitalized

Patients with Severe Community-Acquired Pneumonia and High Inflammatory Response: A Randomized Clinical Trial. *JAMA* 313, 677. doi: 10.1001/jama.2015.88

Vyas, S. C., Mooventhan, A., and Manjunath, N. K. (2019). Effect of hot arm and foot bath on heart rate variability and blood pressure in healthy volunteers. *J. Complement. Integr. Med.* 17, 20180181. doi: 10.1515/jcim-2018-0181

Wang, B., Li, R., Lu, Z., and Zheng, G. (2020). Does comorbidity increase the risk of patients with COVID-19: evidence from meta-analysis. *Aging (Albany N.Y.)* 12 (7), 6049. doi: 10.18632/aging.103000

World Health Organization. (2020). *Coronavirus disease 2019 (COVID-19) technical guidance: laboratory testing for 2019-nCoV in humans*. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance.

Yang, P., Zhao, F., and Wang, G. (2020). Two Cases of Improving Positive results of nucleic acid test in Coronavirus disease 2019 (COVID-19) by induced sputum with atomization inhalation. *Chin. J. Tuberc. Respir. Dis.* 43, E018–E018.

Zhao, F., Sun, S., Xiong, J., and Zheng, G. (2019). The effect of Baduanjin exercise on health-related physical fitness of college students: study protocol for a randomized controlled trial. *Trials* 20, 569. doi: 10.1186/s13063-019-3672-1

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., et al. (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395 (10229), 1054–1062. doi: 10.1016/S0140-6736(20)30566-3

Zou, L., Pan, Z., Yeung, A., Tang, S., Wang, C., Liu, Y., et al. (2018). A Review Study on the Beneficial Effects of Baduanjin. *J. Altern. Complement. Med.* 24, 324–335. doi: 10.1089/acm.2017.0241

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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