The efficacy of mesenchymal stromal cells derived therapies for acute respiratory distress syndrome - a meta-analysis of preclinical trials.

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Research

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Abstract

Background The investigation of Mesenchymal stromal cells (MSC) conditioned medium or extracellular vesicles (exosomes or microvesicles) for acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is a fast growing field in recent years. Our purpose is to investigate these MSC's derived therapies' (MDT) efficacy for ARDS in animal models by meta-analysis.

Methods Meta-analysis of MDT for ALI/ARDS in animal trials. PubMed and EMBASE were searched to screen relevant preclinical trials with a pre-specified search strategy.

Results A total of 17 studies were included in our studies. Compared the control group**,** the pooled result suggested that MDT can reduce lung injury score (mean = -4.02, 95% CI [- 5.28, - 2.23], P <0.0001) and improve survival (OR = -6.45, 95% CI [2.78, 14.97], P <0.0001) significantly. MDT mitigated the infiltration of neutrophils in alveoli (mean = -1.35, 95% CI [-1.69, -1.02], P <0.0001). Also, MDT reduced the wet to dry weight ratio of lung (mean = -2.65, 95% CI [-3.99, -1.31], P= 0.0001) and total protein in BALF (mean = -1.29, 95% CI [-1.63, -0.96], P <0.0001). Furthermore, MDT was found to down-regulate pro-inflammatory mediators such as IL-1, IL-6 and TNF-a, and up-regulate anti-inflammatory mediators such as IL-10.

Conclusion MDT reduced lung injury and improved survival in animal ARDS models as it can ameliorate the lungs' permeability, decrease inflammatory cells infiltration, down-regulate pro-inflammatory mediators and up-regulate anti-inflammatory mediators. However, more animal studies and human trials are needed for further investigation.

Introduction

In critically ill patients, ARDS is a severe clinical syndrome with high morbidity and mortality[1]. The pathophysiological features of ARDS are characterized by diffused alveolar damage, acute non-cardiogenic lung edema, and decreased functional lung volume[2]. Moderate and severe ARDS are usually in need of intubated mechanical ventilation; if exacerbated, a prone position; or, if the patient is unresponsive to regular treatment[3], ECMO as a salvage therapy. While mechanical ventilation can provide the urgently needed respiratory support, it can cause volutrauma, atelectrauma, and biotrauma, which may accentuate the ARDS patients' condition[4]. To date, there are no evidence-based pharmaceutical agents for ARDS, with no treatments directly targeting the pathophysiology of ARDS[5]. Mesenchymal stromal cells (MSC)—a member of the pluripotent stem cells—were first found in bone marrow. With anti-bacterial; immuno-modulatory; and, tissue and organ repair and regeneration characteristics, MSC was widely investigated as a potential therapy in different scenarios for ALI/ARDS in the last few decades[6]. MSC's may be effective for ALI/ARDS caused by a variety of pathogenic factors, as it can ameliorate the lungs' permeability, decrease inflammatory cells infiltration, and down-regulate inflammatory mediators or up-regulate anti-inflammatory mediators. The effects of MSC's were assumed to be due to its engraft and proliferation, which were demonstrated to be rather limited[7]; however, according to current research, the paracrine- and endocrine-related secretome are more important to damage repair[8-10]. MSC may manage intracellular oxidative stress via exosomes, which can be engulfed and re-utilized by macrophages, thus suppressing inflammation and regulating immunity; thereby, it may have potential in lung injury treatment[11, 12]. MSC may be oncogenic, or trigger in patients an immune response which, per se, may exacerbate ARDS. Furthermore, the storage of MSC's may interfere its gene expression or viability. As

extracellular vesicles (EV's) are manufactured from conditioned medium (CM) by centrifugation, in this study, they are collectively referred to as MSC's derived therapy (MDT). MDT that contain these secretome, may have potential in treating ALI/ARDS. Today, MDT-related research is a fast-growing field[9, 13-15]. Although MDT cannot proliferate as MSC's do, they have the advantages of easier preservation and transfer. Furthermore, in comparison with MSC, MDT has subdued immunogenicity, and is an attractive solution for allogeneic transplants[16]. We will investigate the efficacy of MDT for ALI/ARDS, to evaluate whether it can improve survival, lower lung injury severity, and regulate immune balance through meta-analysis in animal models.

Methods

Data sources

PubMed and EMBASE (up to February 14, 2020) were searched to screen relevant preclinical trials with an exquisitely crafted search strategy. Search terms included: acute respiratory distress syndrome, acute lung injury, mesenchymal stem cell, mesenchymal stromal cell, vesicles, microvesicles, exosome, and medium. The search strategy is as follows: ((((((((((vesicles[Title/Abstract]) OR microvesicles[Title/Abstract]) OR ectosomes[Title/Abstract]) OR exosome[Title/Abstract]) OR nanoparticles[Title/Abstract]) OR microparticles[Title/Abstract]) OR exosomes[Title/Abstract]) OR oncosomes[Title/Abstract])) OR medium[Title/Abstract])) AND (((stem cell[Title/Abstract]) OR stromal cell[Title/Abstract]) OR msc[Title/Abstract])) AND ((((Acute Respiratory Distress Syndrome[Title/Abstract]) OR acute lung injury[Title/Abstract]) OR ARDS[Title/Abstract]) OR ALI[Title/Abstract]).

Study Selection

Two authors (FYW and FB) searched and screened the relevant literature independently, then checked the title and abstract of each retrieved article to decide which required further assessment. Full articles were retrieved if the titles and abstracts suggested that the study included a prospective design to investigate the therapeutic effects of MSC derived therapy for ALI/ARDS in animal models. When there were disagreements, the two authors discussed them thoroughly to reach an agreement.

The inclusion criteria: (1) any controlled preclinical studies investigated MSC derived therapy for ALI/ARDS; (2) any animal models, of any species, age, or gender; (3) MSC derived therapy administered with any approach or any dosage. MSC's were defined using the minimal criteria set out in the International Society for Cellular Therapy (ISCT) consensus statement[17, 18].

The exclusion criteria: (1) non-interventional studies were excluded; (2) the data of the study was not extractable for meta-analysis for at least one of the pre-specified outcomes.

Qualitative assessment and Data extraction

Two review authors (WFY and FB) independently extracted data with a customized data extraction form. The risk of bias was assessed by two review authors (FYW and FB). Study characteristics were extracted if they were related to the construct and external validity. The data extraction form included the following detailed information: (1) references and publication date; (2) species of animal; (3) lung injury model; (4) descriptions of the source of MSC; (5) the dose, and route of MSC derived therapy; (6) the time points of assessment.

As the data in the literature were mostly presented as figures and not in numerical form, a validated open source graphical digitizer (WebPlot-Digitizer, version 4.2) was utilized to extract data from figures. First, the images of the figures for relevant outcomes from all included studies were saved as screenshots. Then, these images were uploaded into the program to extract data. The first step was to define the type of graph analyzed, usually a two-dimensional bar plot. Second, the axis was calibrated by assigning four points of known values. Then, the related data points were extracted; they were added by directly clicking on the graph, and WebPlot-Digitizer calculated the precise coordinates of each point, which in turn was used to calculate the mean and standard deviation for each variable.

Data analysis and statistical methods

Data analyses of this review were performed by Review Manager 5.3. A heterogeneity assessment was performed via the $\chi 2$ test, where a p value less than 0.1 was considered as the significant set. A funnel plot was applied to check for publication bias and I2 was applied to estimate the total variation attributed to heterogeneity among studies. Values of I2 less than 25% were considered as having low heterogeneity, and a fixed-effect model for meta-analysis was used. Values of I2 no less than 25% represented moderate (25%-75%) or high levels (>75%) of heterogeneity existing among studies, and a random effects model was applied. All statistical tests were two-sided and a p value of less than 0.05 was considered statistically significant.

Primary and Secondary Outcomes

Our primary outcomes were lung injury score and survival. Secondary outcomes included inflammatory factors IL-1β, IL-6 and TNF-α, anti-inflammatory factor IL-10, lung wet weight to dry weight ratio (W/D ratio), total protein in BALF, and neutrophils in BALF.

Results

The Characteristics Of The Included Studies

All the included studies were conducted over the last few decades. The animal models were mostly established on mice and rats, only one study applied to pigs. Intratracheal administration of LPS or E. coli to induce ALI were the most popular approach among the included studies. Bleomycin, VILI, H1N1 virus, and ischemia-reperfusion were the respective cause of ALI in each other studies. The tissue sources of MSC included bone marrow, umbilical cord, adipose tissue, and neural crest. The dose of MDT also diverged among the studies. Additionally, the outcome assessment time points of studies differed significantly, most of which studies completed within 3 days, while a few lasted up to 1 week. The detailed characteristics of the ones included are presented in **Table 1**.

Reference	Animal, gender	Injury model	MSCs source	Type of stem cell derived therapy	Time of assessment
Amir Varkouhi [19]	SD rats	*E. coli*(5. 109 CFUs), *IT*	Human UC MSC	extracellular vesicles, 100*10^6/kg, IV	48 h after *E. coli* instillation
Antoine Monsel [20]	Male C57BL/6 mice	E. coli (2 or 3×10^6 CFUs), IT	Human BM MSC	microvesicles, 1*10^6 cells/10ul, IT/IV	18, 24, or 72 h after modeling
Chen Wenxia [21]	Male SD rats	BLM(4 mg/kg), IT	Human WJ MSC	microvesicles, IT	48 h or 1 week after bleomycin treatment
Huang Ruoqiong [22]	C57BL/6 mice	LPS(4 mg/kg), IT	Human AD MSC	MSC extracellular vesicles, 100 μg/200ul	48 h after LPS insult
James Devaney [23]	Male SD rats	E. coli(2×109), IT	Human BM MSC	hMSC-CM	48 h after E. coli instillation
Johnatas Silva [24]	C57BL/6 mice	LPS 2 mg/kg, IT	Mouse BM MSC	MSC extracellular vesicles, 1*10^5 cells, IV	24 h after MSCs, or EV administration
Lavinia Ionescu [25]	male C57BL/6 mice	4 mg/kg LPS	Mouse BM MSC	MSC CM 250,000 cells/30μl, IT	48 h post-LPS insult
Li Qing-Chun [26]	Male SD rats	chest trauma induced ALI	Rat BM MSC	MSC-derived Exosomes, 25 ug/100μl, IV	7 days after modeling
Liu Jianpei [27]	Male SD rats	Intestinal IR induced ALI	Rat BM MSC	MSC-derived Exosomes, 5-10 ug/500μl, IV	20 h after modeling
Mahesh Khatri [28]	White-Duroc pigs	H1N1, 5 × 106 TCID50 per pig	Porcine BM MSC	MSC-derived Extracellular vesicles, 10 μg/ml, IT	1 and 3 days post-infection
Mairead Hayes [29]	Male SD rats	Ventilator induced lung injury	Rat BM MSC	MSC-CM, 4 × 10^6 cells/500μl, IV	4 h following VILI induction
Peng Chung-Kan [30]	Male SD rats	IR induced ALI	RAT NC SCs	NCSCs-CM, 5 × 10^6 cells/250μl, IV	90 mins after modeling
Tang Xiaodan [31]	Male C57BL/6 mice	LPS(4 mg/kg), IT	Human BM MSC	MSC microvesicles, 2 × 10^6 cells/30μl, IV	48 h after microvesicles injection
Vincent Su [32]	Male C57BL/6 mice	LPS(5 mg/kg), IT	Mouse MSC	MSC-CM, 200 μl, IV	24 h after MSC-CM treatment
Xu Ning [33]	Male SD rats	Phosgene(8.33 g/m3), inhaled	Rat BM MSC	MSC-derived exosomes, 50 mL, IT	6, 24, and 48 h post-exposure
Yi Xiaomeng [34]	C57BL/6 mice	LPS(1 mg/kg), IT	Mouse BM MSC	MSC-derived exosomes(30 μL), IV	24 h after LPS induction
Zhu Ying-gang [35]	Male C57BL/6 mice	LPS(4 mg/kg), IT	Human MSC	MSC microvesicles, 1.5 × 10^6 cells/ 15 μl	48 h after microvesicles injection

Abbreviations: SD Sprague-Dawley, LPS lipopolysaccharide, CFU colony forming unit, IP intra-peritoneal, IT intratracheal, IV intravenous, BM bone marrow, UC umbilical cord, AD adipose-derived, MSC Mesenchymal stromal cells, CM conditoned medium.

Meta-analysis: MSC derived therapies versus ALI control group.

Primary outcomes: lung injury score and survival

A total of 6 studies investigated the lung injury score. Compared the ALI control group, the pooled result suggested that MDT can reduce lung injury score significantly, mean = -4.02, 95% CI [− 5.28, − 2.23], P < 0.0001, I2 = 67%, (Fig. 2A). 4 studies reported on post-injury survival; in comparison with ALI control group, the synthesis of these results indicate that MDT can promote survival significantly, OR = -6.45, 95% CI [2.78, 14.97], P < 0.0001, I^2 = 2% (Fig. 2B).

Secondary outcomes

Neutrophil level in BALF

The neutrophil in BALF were reported in a total of 11 studies. In comparison with the control group, MDT can mitigate the infiltration of neutrophils in alveoli, mean = -1.45, 95% CI [-1.78, -1.12], P < 0.0001, I^2 = 89%, (Fig. 3A).

Total protein level in BALF

In sum, 12 studies investigated the total protein in BALF. Their pooled result indicates that, compared with control, MDT can ameliorate the protein leakage, mean = -1.29, 95% CI [-1.63, -0.96], P < 0.0001, I^2 = 79%, (Fig. 3B) .

Wet to dry weight ratio of lung (W/D ratio)

The synthetic result of 7 studies proved that MDT can reduce the W/D ratio when compared with the control group, mean = -2.34, 95% CI [-3.42, -1.26], P < 0.0001, I^2 = 74%, (Fig. 3C).

Inflammatory and anti-inflammatory factors pertaining to lung injury.

A total of 5 studies investigated the IL-1 in lung tissue.In these, MDT is shown to decrease the level of IL-1, when compared with the ALI control group, mean = -1.26, 95% CI [− 1.92, − 0.59], P = 0.0002, I^2 = 88%, (Fig. 4A). A sum of 8 studies reported IL-6, and their pooled result suggests that, in comparison with control, MDT can reduce the level of IL-6, mean = − 2.91, 95% CI [-4.41, -1.41], P = 0.0001, I^2 = 85%, (Fig. 4B). Additionally, 7 studies presented data about TNF-a, the synthetic result of which revealed that MDT can down-regulate the level of TNF-a, mean = − 2.48, 95% CI [-3.17, -1.79], P < 0.0001, I^2 = 73%, (Fig. 4C). The pooled result of 8 studies suggested that MDT can up-regulate the level of IL-10, mean = 1.14, 95% CI [0.69, 1.59], P < 0.0001, I^2 = 69%, (Fig. 4D).

Discussion

EV's comprising exosomes and microvesicles are extracted from conditioned medium (CM) through centrifugations; similar to CM, they also possess the MSC's secretome36, 37. Thus, in this study, CM and EV's were integrated as MSC's derived therapies (MDT), the purpose of this study is to summarize the evidence of MDT for ALI/ARDS. To our knowledge, this is the first meta-analysis investigating the efficacy of MDT for ALI/ARDS in preclinical studies.

Our meta-analysis demonstrated MDT can mitigate the severity of ALI/ARDS in animal models. The lung injury score (LIS), a scoring scale under a microscope, is a widely used pathophysiological tool to assess lung injury

severity in preclinical trials. In our study, the pooled result indicated that MDT reduced the LIS significantly—direct evidence that MDT can attenuate lung injury severity. The result also suggested that MDT was able to increase survival in animal trials. Moreover, our study revealed that MDT can down-regulate the levels of inflammatory factors such as IL-1, IL-6 and TFN-a, while up-regulating the level of IL-10, a well-known anti-inflammatory factor. The immuno-modulatory effects of MDT may be an important reason for the ameliorated lung injury and improved survival.

The W/D ratio of lung is an extensively utilized parameter to assess pulmonary vessel permeability in animal studies, which was demonstrated to be decreased in our study. It may indicate that MDT can improve lung water clearance. Our meta-analysis suggests that MDT can down-regulate the infiltration of neutrophils into the alveolar space, which is a feature of infection-induced ALI/ARDS. The decrease of neutrophils in alveoli not only attenuated inflammation and subsequent high vessel permeability in the lung but also reduced lung tissue damage, which in turn may improve the outcomes. In addition, our study discovered that the total protein in BALF was reduced with the treatment of MDT. The reduction of total protein was not just the consequence of down-regulated lung vessel permeability, it also may be the mechanism of improved lung compliance. As protein-rich fluid, called the “hyaline membrane” formed on the alveolar surface, it increased the alveolar interfacial tension and blocked oxygenation in ALI/ARDS.

To date, with regard to treating ARDS, there is no targeted medicine that has proven to be effective[38]. Since 2007, a large body of preclinical trials investigated the efficacy of MSC therapy for ALI/ARDS, which demonstrated that MSC can stabilize the alveolar-capillary barrier, enhance alveolar fluid clearance, and decrease infection and inflammation[39-41]. Micro-vesicles derived from stem cells were reported to contain secretomes, such as protein and mRNA components that are crucial for stem cell renewal and expansion[42]. Since MSC’s were revealed to have potential to treat ALI/ARDS, conditioned medium (CM) or extracellular vesicles (EV’s) of MSC, which possess these secretomes, were studied in recent years[19-35].

ARDS is a common clinical syndrome that causes respiratory distress due to refractory hypoxia for a variety of heterogeneous etiologies. The hallmark of ARDS is non-cardiogenic lung edema, a result of diffused alveolar damage, increased permeability of lung vessels, infiltration of inflammatory cells, and protein-rich fluid leakage to alveolar space, which caused the overwhelming hypoxia[43]. The popularity of lung protective ventilation[44, 45], mainly characterized with low tidal volume and low inspiratory pressure, decreased the ARDS mortality in the early 2000’s[46, 47]. However, in 2013, an RCT discovered that prone positioning can significantly reduce 28-d and 90-d mortality on the basis of lung protective ventilation[48]. The control of driving pressure was also associated with increased survival in ventilator settings in ARDS[49]. Other measures taken for respiratory support, such as lung recruitment and PEEP titration, may increase mortality, thus are not recommended in the clinical routine[50]. Though this increased understanding of ARDS was achieved in the last decades, no pharmaceutical agents were verified to be effective treatments. Trials for medications such as aspirin, intravenous salbutamol, recombinant human keratinocyte growth factor, rosuvastatin, and simvastatin were all ineffective because they didn’t result in reduced mortality of ARDS[5].

MSC’s are an important member of the stem cell family; they adhere to plastic in standard culture conditions, express molecules such as CD73, CD90, and CD105; and, they have the ability of multipotent differentiation—into osteoblasts, adipocytes, chondroblasts in vitro[17, 51]. MSC’s exhibit lung protective potential in numerical basic research via paracrine of growth and anti-inflammatory factors, and down-regulation of inflammatory

pathways. Not only was MSC intensively investigated in vivo and in vitro in preclinical trials, several clinical trials regarding the safety of MSC's for ARDS were also carried out in the past few years[52-54]. Though the safety of MSCs was doubted because of its oncogenic possibility, so far no direct MSCs-related adverse events have been detected in the above trials. The safety of MDT should be more reassuring since no live cells were transplanted during the treatment[55]. According to Katie Famous et al, clinically, ARDS can be divided into two subphenotypes, which have different inflammation status and respond differently to fluid infusion[56]. Whether these distinct ARDS subphenotypes respond differently to MSC or MDT is a topic much worthy of future research.

There are several limitations in our meta-analysis. First, the causes of ALI/ARDS was not unanimous within the studies. Second, the source of MDT was not consistent within the studies, both human origin and animal origin were investigated. Third, the dosage of MDT and the intervention duration were also divergent among the studies.

Conclusion

MDT reduced lung injury and improved survival in animal ALI/ARDS models via the following mechanisms: ameliorating the lungs' permeability, decreasing inflammatory cells infiltration, down-regulating pro-inflammatory mediators and up-regulating anti-inflammatory mediators. However, more animal studies and human trials are needed for further investigation.

Abbreviations

ARDS Acute respiratory distress syndrome; ALI Acute lung injury; LPS lipopolysaccharide; BM bone marrow; UC umbilical cord; AD adipose-derived; MSCs Mesenchymal stem cells.

Declarations

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Authors' contributions

WFY and FB contributed equally to this work, they conceived the idea and analyzed the medical file together. The manuscript was written in English by WFY. QXH and SJS made supportive contributions to this work. ZLX was involved in drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

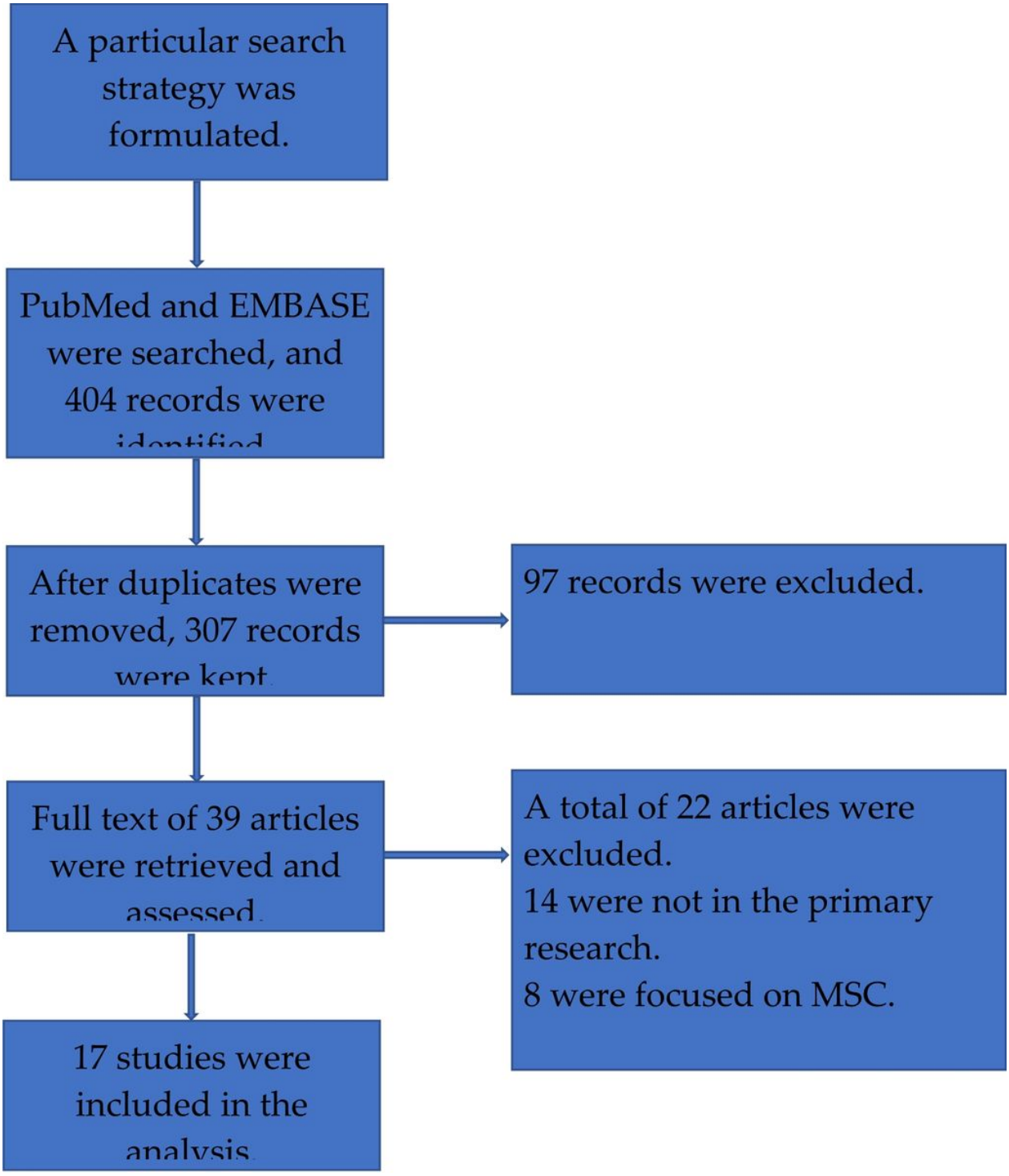

Figure 1

Flow diagram of the literature search process

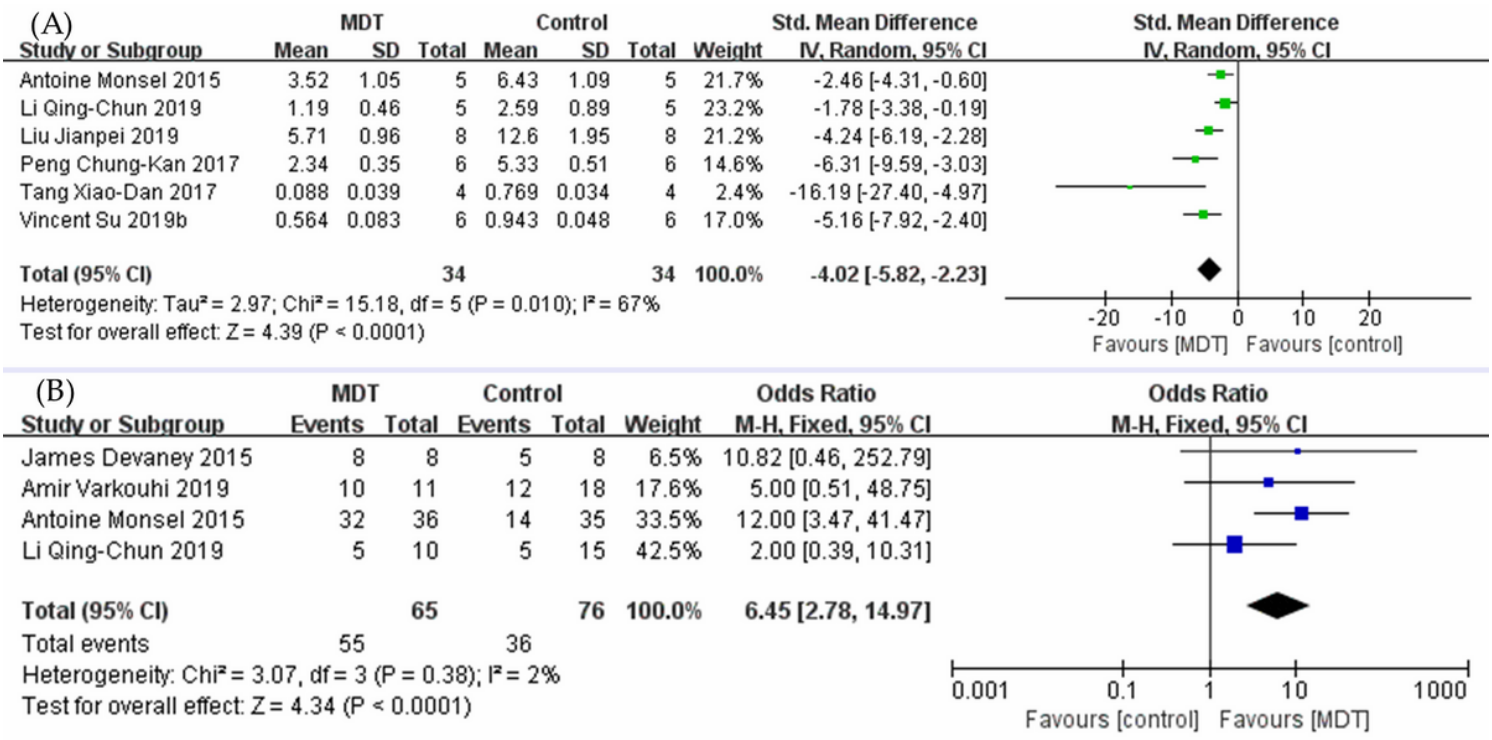

Figure 2

Main outcomes meta-analyses of MDT comparing with ALI control group: (a)lung injury score; (b)survival. The size of each square represents the proportion of information given by each trial. Crossing with the vertical line suggests no difference between the two groups.

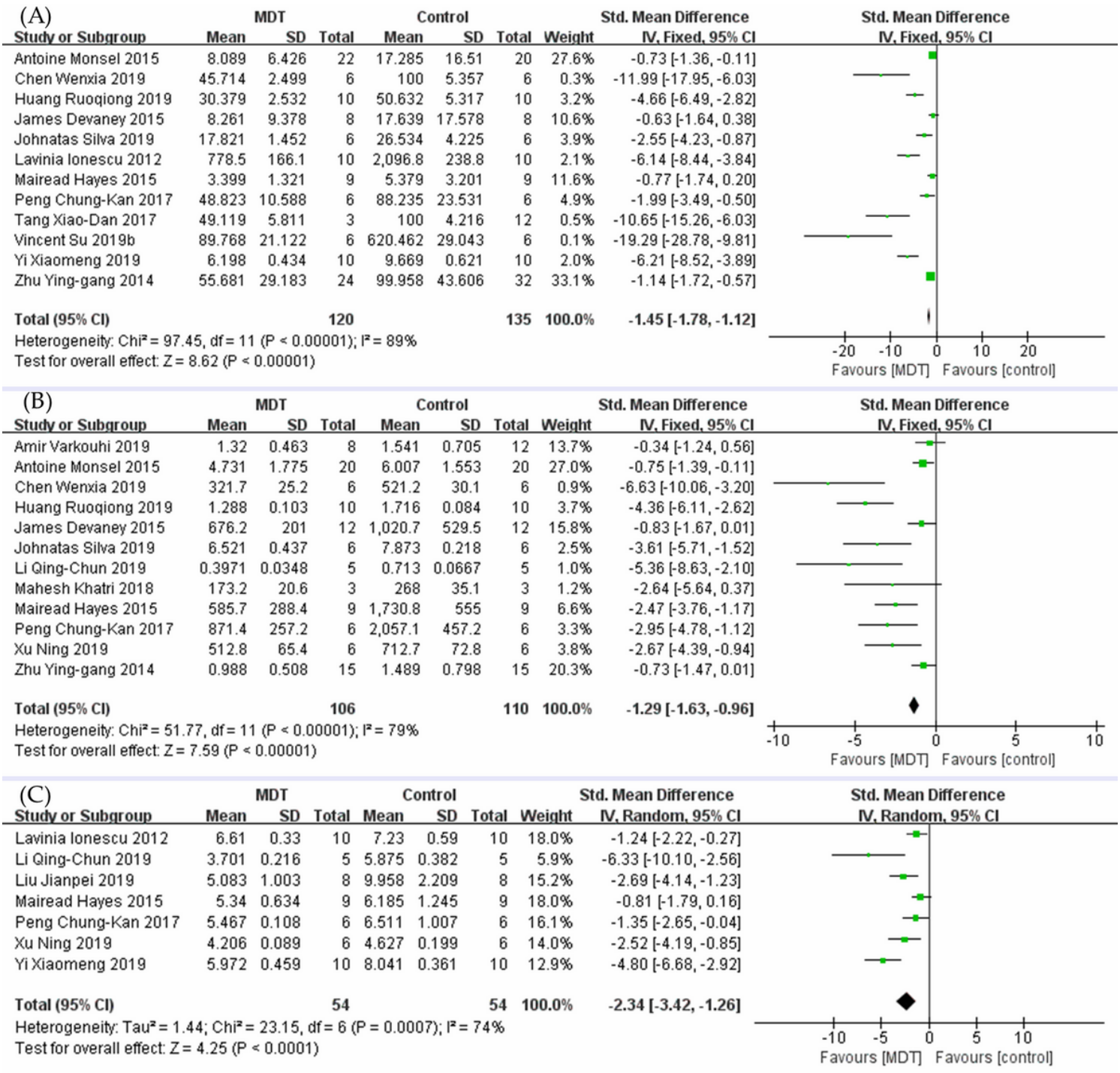

Figure 3

The meta-analyses of neutrophils in BALF(A), total protein in BALF (B) and W/D ratio(C) compare MDT with ALI control group. The size of each square represents the proportion of information given by each trial. Crossing with the vertical line suggests no difference between the two groups. BALF Bronchoalveolar Lavage Fluid.

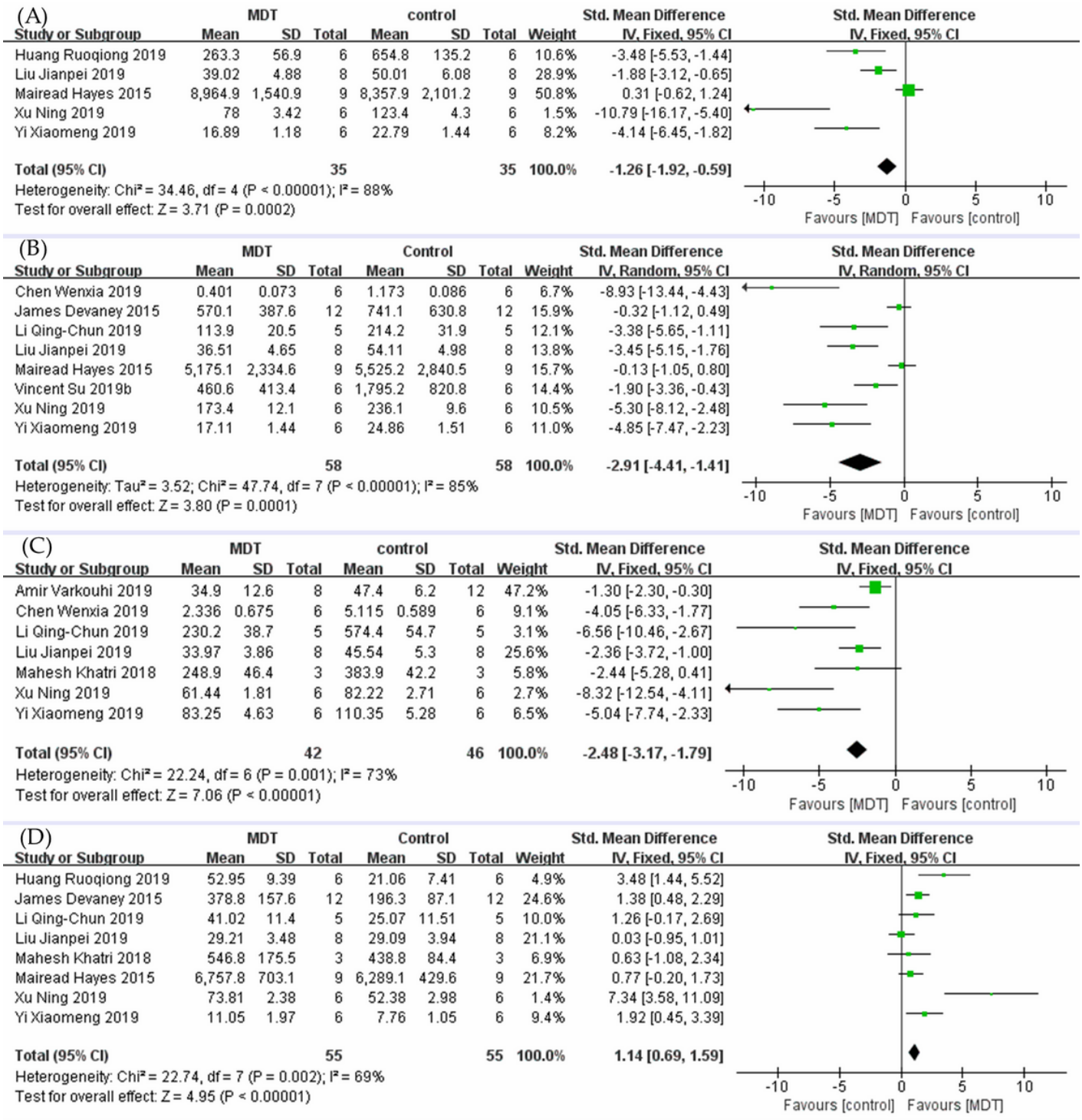

Figure 4

The meta-analyses of inflammatory and anti-inflammatory factors compare MDT with ALI control group: (A)IL-1β, (B)IL-6, (C)TNF-α, and (D)IL-10. The size of each square represents the proportion of information given by each trial. Crossing with the vertical line suggests no difference between the two groups.

Supplementary Files

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