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Research article

Meta-analysis reveals inhibition of the inflammatory cytokine IL-6 affords limited protection post-myocardial ischemia/infarction

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ABSTRACT

Background: Proinflammatory cytokine cascades play crucial roles in the onset and progression of myocardial ischemia and infarction. Clinically, elevated serum levels of pro-inflammatory cytokine interleukin-6 is a poor prognostic indicator for future cardiac events and cardiac morbidity. Despite several reports, there is no clear evidence of cardiac benefits of inhibiting IL-6 in pre-clinical and clinical settings.

Objective: To analyze the available data systematically and perform a meta-analysis to show the evidence of effects of IL-6 inhibition on cardiac remodeling and mortality in ischemic animal models.

Methods: We used PICO framework and the quality of the studies was assessed using SYRCLE's risk of bias tool. Studies with interventions i.e., genetic deletion or pharmacological inhibition of IL-6/IL-6R were included for the meta-analysis. Systematic review was synthesized by including pre-clinical as well as randomized clinical trials involving myocardial infarction patients treated with IL-6 inhibitors. The effect size of the pooled data was determined using standard mean difference and 95% confidence intervals.

Results: A total of 12 pre-clinical studies were included in the review for analysis. Most of the studies showed an unclear risk of bias as the selection and reporting criteria were poorly described. We observed high heterogeneity in the included studies due to the varying duration of myocardial infarction and the dosage of IL-6 antibodies used in the studies. Overall inhibition of IL-6 significantly increased area at risk [$p = 0.001$, SMD = 0.49 (95% CI: -0.36, 1.35)] and significantly reduced ejection fraction [$p = 0.001$, SMD = -0.19 (95% CI: -1.39, 1.01)] and end-diastolic diameter [$p = 0.02$, SMD = -0.25 (95% CI: -0.87, 0.36)] of left ventricle post-MI, but no effects on infarct size [$p < 0.01$, SMD = 0.00; 95% CI: -1.34, 0.58). In randomized clinical trials, the overall effect on C-reactive protein remains significantly unchanged on CRP levels (SMD = -0.38; 95% CI: -1.94, 0.55) post-treatment with IL-6R inhibitor tocilizumab. The meta-regression demonstrates a significant positive correlation ($p = 0.058$) between the increase in ischemic area and duration of ischemia post-surgery in the absence of IL-6. This meta-analysis indicates mixed effect of IL-6 inhibition on cardiac remodeling post-MI, particularly in protecting the myocardium viability from damaging acute inflammation but not significant on cardiac function of ischemic animal models.

Conclusion: Despite the well-established pro-inflammatory nature of IL-6 in myocardial ischemia, our meta-analysis reports a limited contribution of IL-6 in the cardiac remodeling of hearts in animal models of myocardial ischemia. Moreover, genetically deleted IL-6 murine models produced contrasting results. Additional pre-clinical studies exploring the pharmacological inhibition of IL-6R are required to determine the beneficial effects of IL-6 inhibitors in regulating cardiac remodeling. The findings from IL-6R inhibition have better clinical relevance compared to genetically inhibited IL-6.

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1. Introduction

Ischemic heart disease (IHD) is the most prevalent cardiovascular disease which causes the maximum number of deaths across the globe. A recent population-based study revealed about 1.72 percent of the world population is affected by IHD and nine million deaths occur yearly [1, 2, 3]. Myocardial ischemia is a disease condition that is associated with several inflammatory cascades' responses. The onset of acute myocardial ischemia is followed by the release of both pro-inflammatory and anti-inflammatory cytokines which contribute equally towards cardiomyocyte damage and repair mechanisms. Numerous proinflammatory cytokines are released at the damaged site by different cardiac cells and circulating immune cells. The major cytokines facilitating pro-inflammatory response during acute myocardial ischemia are IL-6, IL-1, and TNF alpha [4].

Interleukin-6 is one of the most crucial cytokines that regulate cardiomyocyte survival or apoptosis as well exerts substantial effects on cardiomyocyte remodeling. IL-6 belongs to the interleukin-6 superfamily of cytokines, and other members are IL-11, IL-27, oncostatin M (OSM), cardiotrophin-like cytokine (CLC), ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and cardiotrophin 1 (CT-1) [5]. The activation of IL-6 occurs by binding to the cytokine-specific receptor (IL-6R) and/or signal-transducing protein gp130. Higher plasma IL-6 levels were reported post-myocardial ischemia and in coronary artery disease (CAD). Moreover, elevated intracardiac IL-6 levels were often seen in patients with congestive heart failure (CHF) [6]. Elevated levels of IL-6 post-ischemia have been associated with increased mortality. IL-6 being a pivoting cause of CHF in cardiomyopathy, myocarditis, and left ventricular assist device (LVADs) conditions, circulating levels of IL-6 are associated with the severity of left ventricular dysfunction and are strong predictors of clinical outcomes. Mendelian randomization studies suggested that polymorphism in IL-6 and IL-6R is associated with increased cardiac risk and inflammation [7, 8]. Tocilizumab (TCZ) is an FDA-approved drug that binds to IL-6R and attenuates the inflammation caused by IL-6. Initially, this drug was indicated for the treatment of rheumatoid arthritis patients, and later it was approved for non-rheumatological conditions such as Castleman's diseases, systemic juvenile idiopathic arthritis, giant cell arteritis [9, 10, 11] TCZ is currently undergoing clinical trials against myocardial ischemia; these trials imply that altering the IL-6 levels or suppressing signaling pathways could provide an effective therapeutic approach for CAD.

Over several decades, numerous fundamental and clinical studies have reported mixed results of the effect of IL-6 on the cardiomyocytes, cardiac remodeling, and infarct size in murine models of myocardial ischemia. However, no study has systematically analyzed the effects of genetic deletion or pharmacological inhibition of IL-6 on myocardial ischemia-induced cardiac damage and myocardial remodeling. We asked, whether deleting or inhibiting IL-6 signaling has any effect on cardiac function, structure and mortality? This review aims at understanding the modulation of cardiac remodeling and changes in cardiac function under the pharmacological and genetic inhibition of IL-6 in pre-clinical studies. We conducted a systematic review with meta-analysis to evaluate the effect of IL-6 inhibition on cardiac remodeling in experimental and clinical studies under ischemic conditions. Additionally, we also searched for randomized placebo-controlled clinical trials with IL-6 intervention in ST-segment elevation myocardial infarction (STEMI) and non-STEMI (NTSEMI) patients. The meta-analysis was performed for pre-clinical and clinical studies that satisfied all the inclusion/exclusion criteria.

2. Methods

2.1. Search strategy

This systematic review and meta-analysis protocol have been registered in PROSPERO with registration ID CRD4202121282 (https://www.crd.york.ac.uk/prospero/display/record.php?ID=CRD42021212828). Various electronic databases were searched to identify studies that examined the effects of IL-6 inhibition on cardiac remodeling in myocardial ischemia/infarction. This review was prepared according to the Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA) statement. Literature searches were conducted to identify pre-clinical studies investigating the effects of IL-6 on cardiac remodeling post-myocardial infarction. The search was performed using PubMed, Medline, Google Scholar, Scopus and Cochrane library, conducted till May 31, 2021. The following search terms were used ("IL-6 inhibition") AND ("Myocardial Infarction") OR ("Myocardial ischemia", OR ("Cardiovascular Diseases/diagnosis") AND ("Mice/Rat") ("IL-6R inhibition") AND ("Myocardial Infarction") AND ("Clinical studies") ("IL-6 inhibition", [Text word]) AND ("Myocardial Reperfusion Injury" [Mesh]) OR ("Cardiomyopathies", [MESH] AND ("Mice/Rat", [text word]) ("IL-6 genetic knockout", [Text word]) AND ("Myocardial Infarction" [Mesh]) OR ("Myocardial ischemia", [Mesh] OR ("Cardiovascular Diseases/diagnosis", [Mesh]) AND ("Mice/Rat", [text word]) ("Anti-IL-6 antibody", [Text word]) AND ("Myocardial Infarction" [Mesh]) OR ("Myocardial ischemia", [Mesh]) OR ("Coronary artery diseases/diagnosis", [Mesh]) AND ("Ventricular Remodeling/drug effects", [Mesh]) ("Mice/Rat", [text word]) (Supplementary Table 1). Following the search, the resulting records were then screened by two independent investigators to determine the eligibility of the study for the systematic review and meta-analysis. Studies that included myocardial ischemia animal models with IL-6 systemic knockout or pharmacological IL-6 inhibition as intervention, were considered as eligible studies for pre-clinical analysis. Clinical studies including patients with myocardial infarction (MI) treated with antibodies against IL-6 or IL-6R monoclonal antibodies are eligible for inclusion. Predetermined primary endpoints for our meta-analysis comprised of survival rate, cardiac remodeling, infarct size, area at risk as well as the secondary outcomes that included echocardiographic measurements and hemodynamic parameters. Two investigators then independently screened published studies comprising mentioned outcomes and excluding those that failed to determine at least one of the mentioned endpoints.

2.2. Inclusion, exclusion criteria and data extraction

We followed the PICO framework for the selection of studies for our analysis. Our population of interest was mouse and rat studies for the pre-clinical data analysis and human patients diagnosed with MI for clinical studies. For inclusion criteria, the intervention included studies with genetic and pharmacological inhibition of IL-6. The studies on mice with IL-6 gene knockout or IL-6R gene knockout with the induction of myocardial infarction (coronary artery ligation, permanent ligation of left anterior descending coronary artery, Ischemia and reperfusion and apical resection) were included. Pharmacological inhibition of IL-6 by using anti-IL-6 or anti-IL-6R monoclonal antibodies concomitantly with the induction of myocardial infarction was also included. Human placebo-controlled randomized trials (RCTs) involving patients with MI (STEMI and NSTEMI) with a short-term or long-term dosage of IL-6 or IL-6R antibodies were eligible and included in the analysis. All timings, dosage and frequency of treatment were appropriately considered for both pre-clinical and clinical studies. For the meta-analysis, in the comparator group, we included studies that contained the following criteria: Sham, wildtype mice, vehicle or isotype treated (control IgG) mice. In the included clinical studies, all the healthy individuals were considered as control groups. Studies with separate control groups were considered as suitable for inclusion in the systematic review only.

Two investigators (SD, MA) separately screened the studies with mentioned inclusion criteria from all the collected studies. The following pre-determined data points were extracted into self-designed excels files: a surgical procedure performed to achieve the ischemia in mice models, duration of infarction, age and weight of the animals, number of animals included in each group (Sham, MI, MI + IL-6 knockout), survival

percentage compared to controls, heart rate and heart weight/body weight, percent change in infarct size and area at risk, cardiac function (ejection fraction and fraction shortening), echocardiographic and hemodynamic parameters: left ventricular end-diastolic diameter (LVEDD), Left ventricular end-systolic diameter (LVESD), rate of rise of left ventricular pressure (dP/dT max and dP/dT min). In the pharmacologically inhibited group, the following data points were collected: method of drug administration, dosage and duration of treatment. From human studies, serum CRP levels, cardiac Troponin-t levels and IL-6 levels were extracted. Data provided in study tables or figures were collected as mean and standard deviation (SD) for both control and experimental groups for each endpoint. The data provided as median and percentile were converted to mean and SD using appropriate formulas as mentioned in Xiang Wan et al.2014 [12]. The data presented graphically was quantified using webplotdigitizer platform. For incomplete data, the corresponding authors were contacted via email to provide supplementary useful information.

2.3. Study quality assessment

We checked the quality of the included randomized controlled trials using "risk of bias" tool according to the Cochrane Handbook (chapter 8:Assessing risk of bias in a randomized trials) [13]. Sequence generation, allocation concealment, blinding of participants and personnel, selective reporting were assessed, each study were graded as "yes (+)", "no (-)", "unclear (?)", which considered as low risk, high risk, and uncertain risk of bias respectively.

We checked the quality of the studies using SYRCLE's risk of bias tool as described in Hooijmans et al.2014 [14]. Two investigators (SD, MA) independently answered all the questions mentioned in the ROB tool for animal studies, a derivative of the Cochrane risk of bias tool that evaluates for clinical studies. In detail, the following questions are 1. Was the sequence randomly generated and applied? 2. Were the baseline characteristics of the animals mentioned? 3. Was the allocation effectively concealed? 4. Were the random housing of the animals followed? 5. Were the investigators blinded with the animal's intervention during the experiment? 6. Were the animals selected randomly for outcome assessment? 7. Whether the reasons for the exclusion of the animals mentioned? 8. Were mentioned studies free from selective reporting? 9. Were animal welfare regulations followed for the experiment? For each bias parameter, the investigators answered yes which indicates "low risk", of bias, no for "high risk", of bias and "unclear", indicates when the risk of bias was not mentioned clearly. Disagreements in the quality assessment were resolved by discussing with the principal investigator.

2.4. Data analysis and statistics

A total of eleven studies were selected for meta-analysis as all of them satisfied the inclusion criteria. Data were analyzed using Stata/SE 15 (StataCorp, College Station, Texas). Meta-analysis was only performed if there were more than 3 independent studies for the given outcome. Due to varying surgical procedures and outcome measurements, we used a random-effects model for the analysis with the Der Simonian-Laird estimator for each outcome. We performed meta-regression using linear prediction model in Stata to examine the change in infarct size in the absence of IL-6 with the duration of ischemia post-LAD surgery. The effects size was measured using standardized mean difference (SMD) and its 95% confidence interval (95% CI). For each endpoint, we measured SMD, 95%CI, heterogeneity which were represented as I^2 and p-values. If studies have multiple control groups, the one which follows the same surgical procedure as the experimental group was selected. For all the selected studies mean and standard deviation values were extracted, and if the SD values were not mentioned for any given endpoint, then SD values were calculated from the provided mean data.

3. Results

3.1. Study selection

From the initial search, a total of 18,397 records were collected from all the databases. A total of 11,631 records remained after the removal of duplicated records. After the initial screening 10,721 from title selection and 700 records from review articles, conference papers and book chapters were excluded from the study. In the second screening two investigators (SD, MA) independently screened 210 studies and excluded 156 studies because they lacked to provide animal studies with IL-6 intervention and clinical studies that did not include MI patients and other case studies and crossover studies. A total of 54 eligible full-text studies were assessed out of which 35 records were unsuitable due to the following reasons: non-ischemic model and studies with outcomes not of interest (Figure 1). Finally, 12 pre-clinical and 7 clinical studies were included for qualitative synthesis and 12 experimental studies met all the inclusion criteria for meta-analysis.

3.2. Study characteristics

The characteristics of the included pre-clinical studies are summarized in Table 2. Among these studies left anterior descending artery (LAD) was the most commonly used ischemia model, however a few studies also employed coronary artery ligation or coronary occlusion models. The majority of the studies were performed in genetic deletion animal models, whereas pharmacologically inhibited models were used in only four studies. Although, the maximum number of studies utilized male mice only one study failed to define the sex ratio as the surgical procedures were carried out on neonatal mice. In all the included studies mice were provided with ab libitum feed and water. All these animals were between the age of 8 and 16 weeks, with an exception in the case of Tang et al. 2018. Under selection bias, one study reported randomization, most of them failed to report while in a few the process was unclear. The performance bias remained unclear in most of the experimental studies (Figure 2). All studies that regulated compliance with animal welfare and other biases are summarized in Table 1. In clinical trials, all the studies were double blinded, randomized, placebo-controlled trials. Blinding of the outcome measurement was unclear in all the trials (Figure 3). All the trials included STEMI and NSTEMI patients and the female to male ratio was very low in all of them. The other characteristics are summarized in Table 3.

3.3. Meta-analyses

3.3.1. Clinical trials-

After the thorough search, a total of 7 randomized controlled trials were included in the study. However, out of 7 published trials 4 were from the same clinical trial (NCT01491074) which measured different outcomes. We included 3 double blinded, randomized clinical trial for meta-analysis. The common parameter measured across all these trials were CRP, Tnt levels, adverse events and serum IL-6 post treatment. Many studies have reported IL-6 as a major inducer of CRP, and high CRP indicates unfavorable outcomes in acute myocardial ischemia. Therefore, high sensitivity C-reactive protein were measured as the primary outcome post treatment with tocilizumab at different time points. The random effect model reveals slight decrease in the CRP levels [$p = 0.44$, SMD = 0.38 (95% CI: -1.34, 0.58)] (Figure 4A) in TCZ group compared to the control. Similarly, Tnt levels measured across 2 trials shows slight decrease in the TCZ treated group [$p = 0.08$, SMD = -0.27 (95% CI: -0.58, 0.04)] (Figure 4B) compared to placebo group. These reports suggest that inhibiting the IL-6 pathway effectively reduces the CRP and TnT levels after acute MI.

Major adverse cardiac events (MACE) are crucial endpoints and set the tipping points of success in clinical settings. Out of 7 published clinical trials, 3 studies recorded adverse events as primary endpoints.

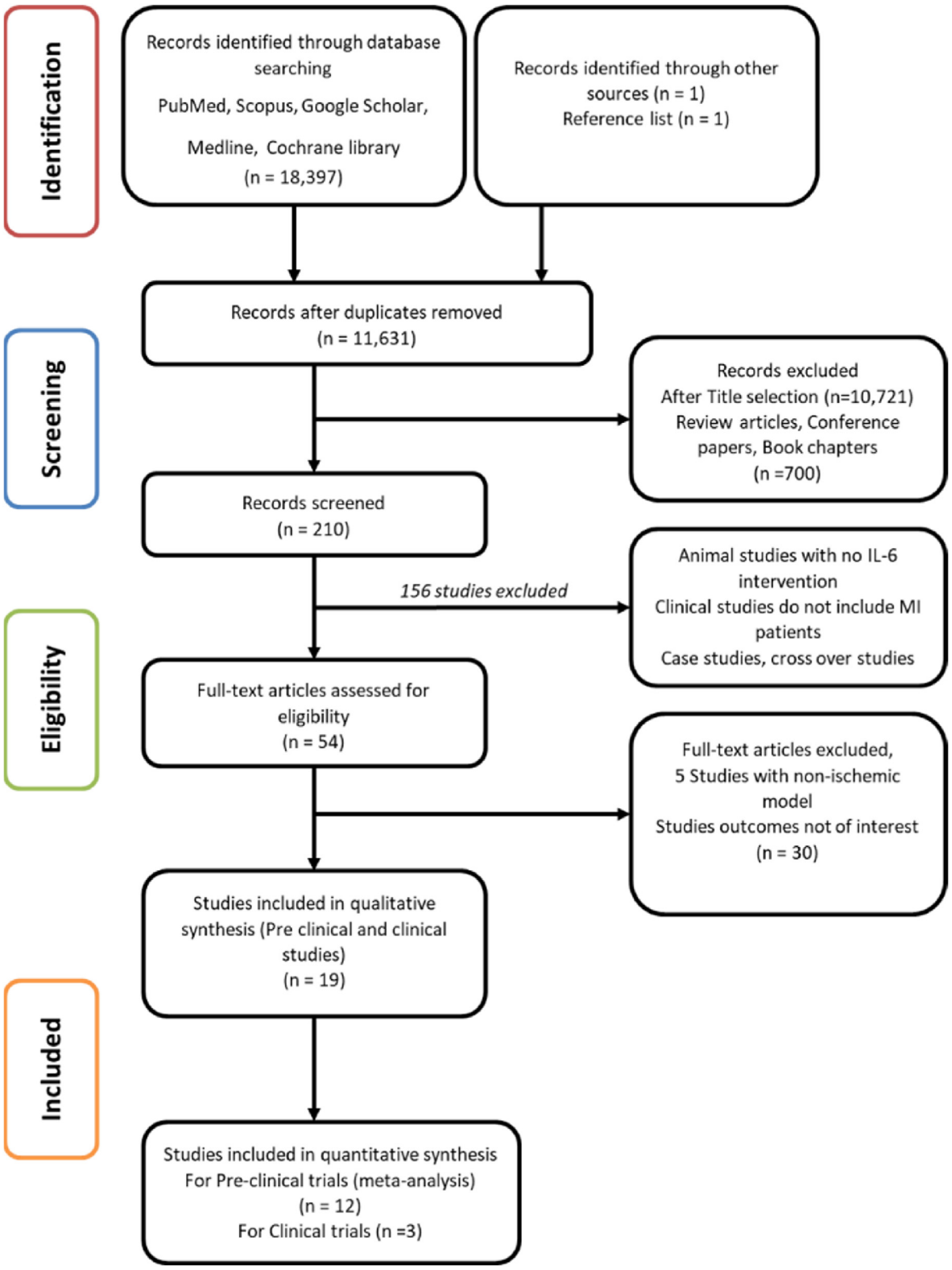


Figure 1. A PRISMA flow diagram of selected studies.

Both the parameters such as recurrent myocardial ischemia and cardiac events did not find significant difference in the incidence of adverse events. The overall random effect measured showed low heterogeneity ($I^2 = 15.30\%$) among the studies. The pooled effect shows no increase in the adverse event post treatment with TCZ as represented by relative risk [p = 0.54, RR = 0.03 (95% CI: -0.01, 0.07)] (Figure 4C). The echocardiographic results were measured as secondary outcome in 2 trials. In the Kleveland et al. 2016 (NCT01491074), NSTEMI patients were administered with a single dosage of 280 mg TCZ and post 6 months follow-up the left ventricular inner diameter was non-significant for between-

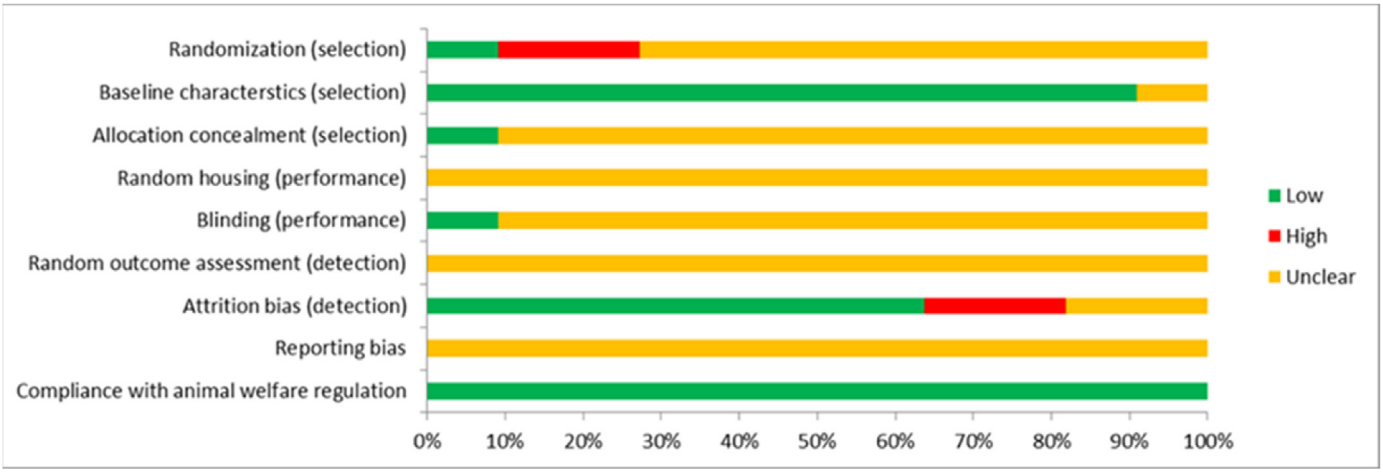


Figure 2. Risk of bias summary graph reporting average of study qualities of the included studies. The Y-axis of the graph indicates the risk of bias and the X-axis shows study percentages.

Table 1. Risk of Bias summary table (✓ = yes, × = No, UC = unclear).

Study	Selection bias			Performance bias		Detection bias				Total Score (Max 9)
	Randomization	Baseline characteristics	Allocation concealment	Random housing	Blinding	Random outcome assessment	Attrition bias	Reporting bias	Compliance with animal welfare regulation	
Jing et al.2019 [15]	✓	✓	✓	UC	×	UC	UC	UC	✓	4
Fuchs et al.2003 [16]	×	✓	UC	UC	×	UC	×	UC	✓	2
Tang et al.2018 [17]	×	UC	UC	UC	×	UC	✓	UC	✓	2
Muller et al.2014 [18]	×	✓	UC	UC	×	UC	×	UC	✓	2
Jong et al.2016 [19]	UC	✓	UC	UC	×	UC	UC	UC	✓	3
Bonda et al.2014 [20]	UC	✓	UC	UC	×	UC	✓	UC	✓	3
Dawn et al. 2004 [21]	×	✓	UC	UC	×	UC	✓	UC	✓	3
Hartman et al.2016 [22]	×	✓	UC	UC	✓	✓	✓	UC	✓	4
Kobara et al.2010 [23]	×	✓	UC	UC	×	UC	✓	UC	✓	3
Kaminski et al.2009 [24]	×	✓	UC	UC	×	UC	✓	UC	✓	3
Hilfiker-Kleiner et al.2010 [25]	×	✓	UC	UC	×	UC	✓	UC	✓	3
George et al.2021 [26]	×	✓	UC	UC	×	UC	×	UC	✓	2

group differences in changes from baseline [27]. Likewise, in the second trial (NCT03004703) Broch et al. 2021, the baseline adjusted left ventricular volume showed no between group differences (p = 0.54) [33]. Percentage change in the left ventricular ejection fraction was also measured in these trails and post TCZ treatment showed no significant between-group differences since hospitalization and 6 months follow-up [27, 33].

3.3.2. Mortality

A total of 12 studies were included for meta-analysis, out of those, 5 studies (151 controls and 141 IL-6 deleted/inhibited mice) mentioned the survival rate compared to their respective control groups. All the studies analyzed post-myocardial ischemia survival in percentage and we used the mortality percent from the given survival data. All the studies analyzed post-myocardial ischemia survival in percentage and we calculated mortality percentage from Kaplan Meier analysis from the given survival data. After pooling the results and employing a random-effects model, it revealed no significant change in mortality rate in IL-6 KO and IL-6R antibody-treated mice [p = 0.82, RR = 0.05 (95% CI: -0.40, 0.51)] (Figure 5).

3.3.3. Cardiac remodeling: infarct size, area at risk and cardiomyocyte cross-sectional area

Out of 12 studies analyzed, 8 studies assessed infarct sizes ((106 controls and 123 IL-6 deleted/inhibited animals) and 6 studies (52 controls and 62 IL-6 deleted/inhibited animals) reported area at risk

Table 2. Characteristics of included experimental studies (LAD- Left anterior descending coronary artery; KO- Knockout; AAR- Area at risk, CSA- Cross-sectional area; WT- Wildtype; IV- Intravenous, IP- Intraperitoneally).

Studies	Animal model	Age (wks)	weight	MI Surgical procedure	Duration of MI	Dosage/Method of administration	Outcome measures
Jing et al. 2019 [15]	IL-6 KO Mice	12–16	-	LAD	28 days		Survival rate, echocardiographic outcomes, Fibrosis
Fuchs et al.2003 [16]	IL-6 KO Mice	11–14	25–30g	LAD	6 weeks		Survival rate, infarct size, AAR, myocyte CSA, echocardiography measurements
Tang et al.2018 [17]	IL-6 KO Mice	-	Neonatal mice	apical resection	4 weeks		Echocardiographic measurements, fibrosis
Muller et al.2014 [18]	WT Mice	16-18	-	LAD	72h and 3 weeks for ventricular function, 24h for infarct size and AAR	Anti-IL6 Ab (250ug/mouse)/IP dosage	Survival rate, infarct size, myocyte CSA, echocardiographic measurement
Jong et al.2016 [19]	IL-6 KO Mice	8–10	-	surgical ischemia in closed-chest	1h ischemia and 0,1/2, 3 and 24h reperfusion		Infarct size, AAR and Fibrosis
Bonda et al.2014 [20]	IL-6 KO Mice	12-14	-	LAD	8 weeks		Echocardiographic measurements
Dawn et al.2004 [21]	IL-6 KO Mice	12–26	25–35g	coronary occlusion	30-min coronary occlusion followed by 24 h of reperfusion		Infarct size and AAR
Hartman et al.2016 [22]	WT Mice	8	26–29 g	LAD	28 Days	Anti-IL-6R Ab* (MR16-1 2mg/mouse)/IV dosage	Infarct size, AAR, Fibrosis, hemodynamic measurements
Kobara et al.2010 [23]	WT Mice	8–12	25–30g	LAD	7 Days and 28 Days after surgery	Anti-IL-6R Ab# (MR16-1 500ug/body)/IP dosage	Survival rate, infarct size, AAR, Fibrosis, myocyte CSA, echocardiography measurements
Kaminski et al.2009 [24]	IL-6 KO Mice	12–16	27–29g	LAD	30 min of regional reversible myocardial ischemia		Infarct size and AAR
Hilfiker-Kleiner et al.2010 [25]	gp130 KO mice	12-16	-	LAD	2 weeks		Echocardiographic measurements, Myocyte CSA
George et al.2021 [26]	Male Sprague-Dawley rats	7-8	200–250g	LAD for 50min	50 min of ischemia, followed by reperfusion	Anti–IL-6-Ab (0.1 μg/mg)/IP sgp130Fc (0.5 μg/mg)/IV	Infarct size, AAR, cardiac magnetic resonance imaging

(* indicates MR16-1 dosage (anti-mice IL-6R antibody) of 2mg/mouse intravenously; # represents MR16-1 dosage (rat anti-mouse IL-6R monoclonal antibody) of 500ug/body intraperitoneally).

(AAR), and both the morphological features were measured in percentage. The included studies reported infarct size as a percentage change between LAD-ligated and sham-operated groups with or without IL-6 deletion/inhibition. The pooled data from 8 studies reported no significant changes in the infarct size in experimental group compared to control group [$p < 0.001$, SMD = 0.00; 95% CI: -1.02, 1.03)] (Figure 6A). However, in contrast to infarct size, data sets from 6 different studies reporting AAR showed significant increase in IL-6 inhibited groups compared to controls [$p = 0.001$, SMD = 0.49 (95% CI: -0.36, 1.35)] (Figure 6B). Also, heterogeneity was observed in both the outcomes, respectively ($I^2 = 90.54\%$ and $I^2 = 79.53\%$), we performed subgroup analysis based on the IL-6 inhibitory model used in the studies. Subgrouping of the studies into genetic and pharmacological inhibition failed to improve heterogeneity in infarct size. However, in AAR the subgrouping revealed low heterogeneity in genetic ($I^2 = 11.32\%$) and pharmacological inhibited studies ($I^2 = 0.00$).

Furthermore, a total of 8 studies (85 controls and 95 IL-6 deleted/inhibited mice) measured the change in cross-sectional area (CSA) of cardiomyocyte and fibrosis post-infarction between control and IL-6 inhibited/deleted groups. All the studies analyzed the cardiomyocyte CSA in square micrometers and reported fibrosis as the change in percentage compared to control. The cumulative data from studies revealed a slight decrease in the total fibrosis in the IL-6 group (IL-6 knockout/IL-6R antibody) compared to the controls [$p = 0.80$, SMD = -0.70 (95% CI: -1.94,0.55)] (Supplementary Figure 1). Similarly, a reduction in the cardiomyocyte CSA was observed in the IL-6 group compared to the wild-type controls (Table 4).

3.3.4. Cardiac function: echocardiographic and hemodynamic parameters

To evaluate the cardiac function in the absence of IL-6 post-MI, we selected the following echocardiographic and hemodynamic measurements: ejection fraction (EF), fractional shortening (FS), left ventricular end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), rate of contraction (the maximal rate of rise of left ventricular pressure: dP/dT_{max}) and rate of relaxation (dP/dT_{min}) of ventricular pressures, and heart rate in the pre-clinical settings. The EF and FS were expressed as percentages in 8 and 6 studies, respectively. Based on the random-effects model, our analysis showed an improvement in FS in the absence of IL-6 post-MI compared to wild types with effect size [$p = 0.07$, SMD = 2.38 (95% CI: -0.15, 4.91)] (Figure 7B). Nevertheless, the ejection fraction remained significantly unaffected from the pooled studies [$p = 0.001$, SMD = -0.19 (95% CI: -1.39, 1.01)]. However, there was significant heterogeneity between the studies (Figure 7A, B). To further estimate the LV function, studies evaluating LVEDD, LVESD in millimeters, or relative volume units (LV- diastolic and systolic volume) and dP/dT_{max}, dP/dT_{min} assessed in mmHg/sec were considered for analysis. The standard mean difference and pooled effect size were calculated for each outcome if the number of studies was more than three (Table 4) (Supplementary Figure 2). The pooled data stated a significant minor decrease in LVEDD [$p = 0.02$, SMD = -0.25 (95% CI: -0.87, 0.36)] and an increase in the LVESD [$p = 0.11$, SMD = 0.42 (95% CI: -0.12, 0.96)] (Figure 8A, B). However, the difference was not statistically significant between the groups for LVESD. We also observed a decrease in the heart rate in the experimental group [$p = 0.06$, SMD = -0.69 (95% CI: -1.42, 0.03)].

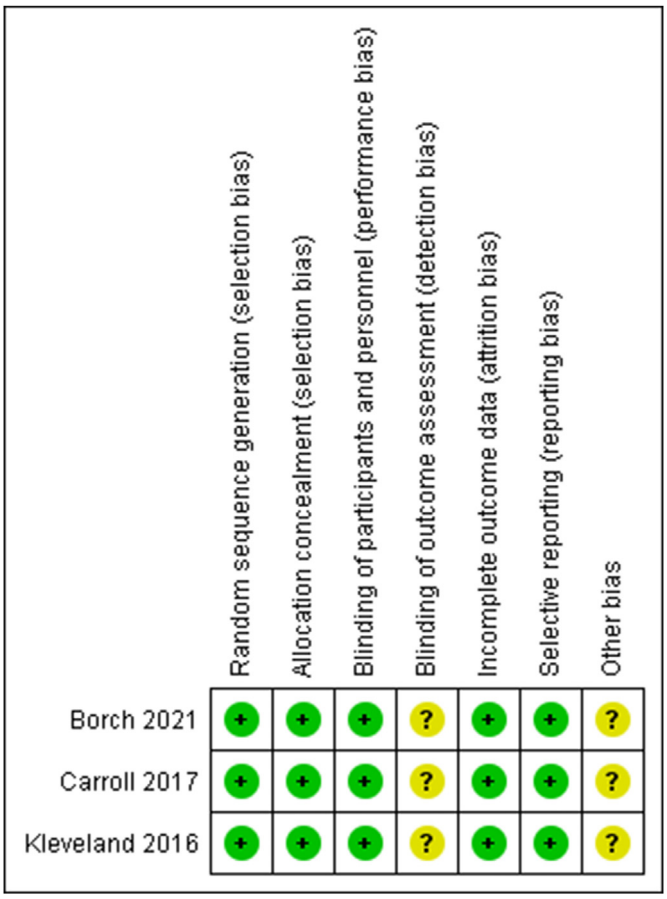


Figure 3. Quality of included randomized controlled trials. "+", "-", "?", indicates low risk, high risk and unclear risk of bias.

3.3.5. Inflammatory signatures post-MI and IL-6

IL-6 plays an important role in inflammatory pathways. In this review, we included the following parameters to evaluate the response of inflammation in the absence of IL-6: neutrophils infiltration, and changes in the serum IL-6 levels. A total of 5 studies (3-pre-clincal and 2 clinical) were included to analyze these parameters, which comprised 52 number of IL-6 inhibited and 34 control animals. The neutrophil cell count was decreased in the infarct area in the pharmacological IL-6 inhibited animals post infarction [p = 0.47, SMD = -2.37 (95% CI: -3.25, -1.9)]. Overall, we observed a significant decrease in the infiltration of neutrophils under IL-6 inhibition (Figure 9).

After myocardial ischemia, several studies have reported increased levels of IL-6 and the same was observed in all of the included studies [34, 35]. In the myocardial ischemic animal model, the IL-6 protein levels were increased compared to the sham model (Supplementary Figure 2) [p = 0.00, SMD = 5.66 (95% CI: 3.25,8.06)]. Pharmacological inhibition of IL-6R resulted in a 4 times increase in the plasma IL-6 levels in the IL-6R Ab treated mice compared to the IgG group [22]. Correspondingly, a pronounced increase in the IL-6 levels was reported in MI patients administered with TCZ compared to placebo by the end of the third day during hospitalization (Supplementary Table 3.) [27].

3.4. Publication bias

We conducted publication bias studies for primary endpoints-infarct size, ejection fraction. The publication bias analysis for other endpoints was not performed due to the small number of studies. Funnel plot of primary outcome infarct size and ejection fraction revealed no asymmetry in the included studies. Egger's regression test suggests no small study effect in both change in infarct size and ejection fraction (Figure 10A, B) [36]. We also performed Nonparametric trim-and-fill analysis of publication bias with linear estimator that reported zero imputed studies for all the outcomes.

3.5. Meta-regression

As we observed high heterogeneity in the overall change of infarct size with the duration of the ischemia which suggests the influence of time post-ischemia, we performed a meta-regression for these characteristics. Regression analysis demonstrated that in the absence of IL-6 the change in infarct size tends to positively correlate with the duration of ischemia post- LAD surgery (Figure 11). Additionally, the regression

Table 3. Characteristics of included clinical trials (TCZ- Tocilizumab; STEMI- ST-segment elevation myocardial infarction; NSTEMI- Non ST-segment elevation myocardial infarction; M:F- Male: Female Ratio, hs-CRP- high sensitivity C-reactive protein, TnT- Cardiac Troponin-T).

Trial	Source	Gender (M:F)		Age, y mean (SD) or median (range)		Patients	Population	Drug dose	Duration	Outcome measures
		Placebo	TCZ	Placebo	TCZ					
NCT01491074	Kleveland et al. 2016 [27]	19:1	10:1	60.1 ± 9.9	59.8 ± 7.7	NSTEMI	117	280mg of TCZ/ intravenously	6 months	hs-CRP, hs-TnT, IL-6 levels, Serious adverse effects, Neutrophil count, cardiac function and Heart failure
NCT02419937	Carroll et al. 2017 [28]	14:2	10:2	67.7 ± 9.5	70.7 ± 10	STEMI and NSTEMI	28	162mg of TCZ/ subcutaneously	30 days	Major adverse cardiac events, CRP levels
NCT01491074	Ueland et al. 2018 [29]	19:1	10:1	60.1 ± 9.9	59.8 ± 7.7	NSTEMI	117	280mg of TCZ/ intravenously	6 months	Seum Lp(a) measurements
NCT01491074	Orrem et al. 2018 [30]	19:1	10:1	60.1 ± 9.9	59.8 ± 7.7	NSTEMI	117	280mg of TCZ/ intravenously	6 months	Expression of anaphylatoxin receptor, PBMC count
NCT01491074	Kleveland et al. 2018 [31]	19:1	10:1	60.1 ± 9.9	59.8 ± 7.7	NSTEMI	117	280mg of TCZ/ intravenously	6 months	Plasma levels of interferon gamma-inducible protein (IP-10) and macrophage inflammatory protein-1β
NCT01491074	Holte et al. 2016 [32]	0 female	32:1	59.3 ± 9.5	57.8 ± 6.1	NSTEMI	42	280mg of TCZ/ intravenously	6 months	Coronary flow reserve measurements
NCT03004703	Broch et al.2021 [33]	4:1		62 ± 10	60 ± 9	STEMI	200	280mg of TCZ/ intravenously	6 months	Myocardial salvage index measured by cardiac MRI (CMR), include final infarct size measured by CMR and hs-CRP, hs-TnT levels.

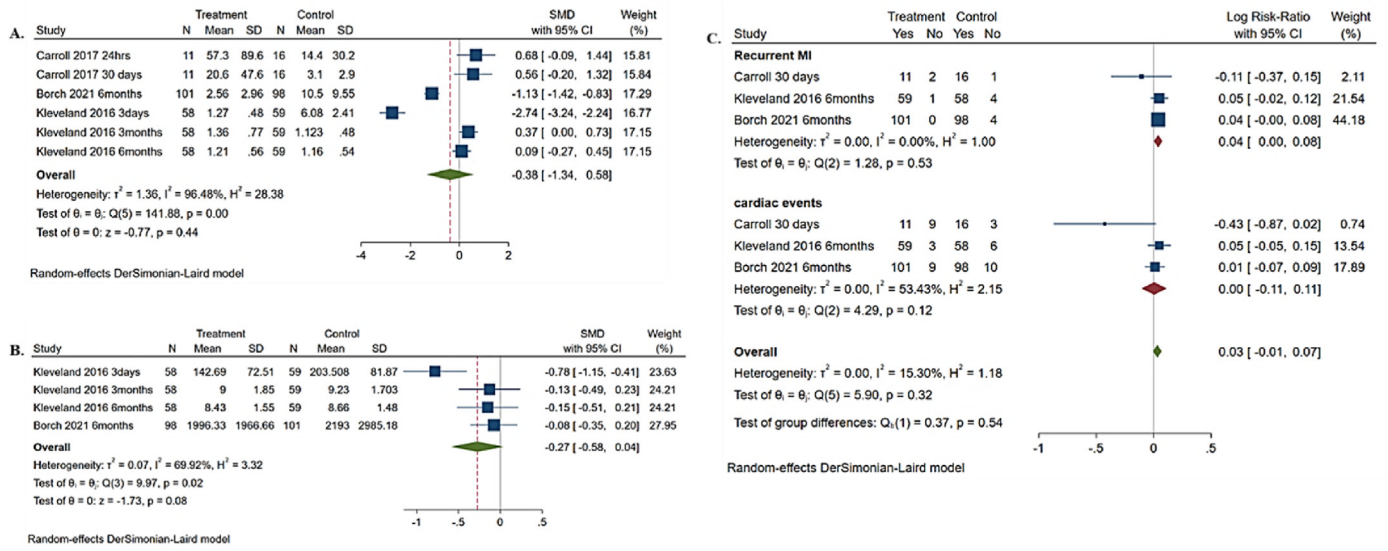


Figure 4. Quantitative analysis of studies estimating the effects of IL-6 inhibition on CRP and Tnt levels and adverse effects: Forest plot of studies measuring (A.) CRP levels and (B.) Forest plot of studies assessing Tnt levels using random-effect model (SMD, 95% CI) (C.) Adverse events in patients with MI using random-effect model (logs Risk ratio, 95% CI). The plot shows individual and pooled SMD along with the relative weight of each study.

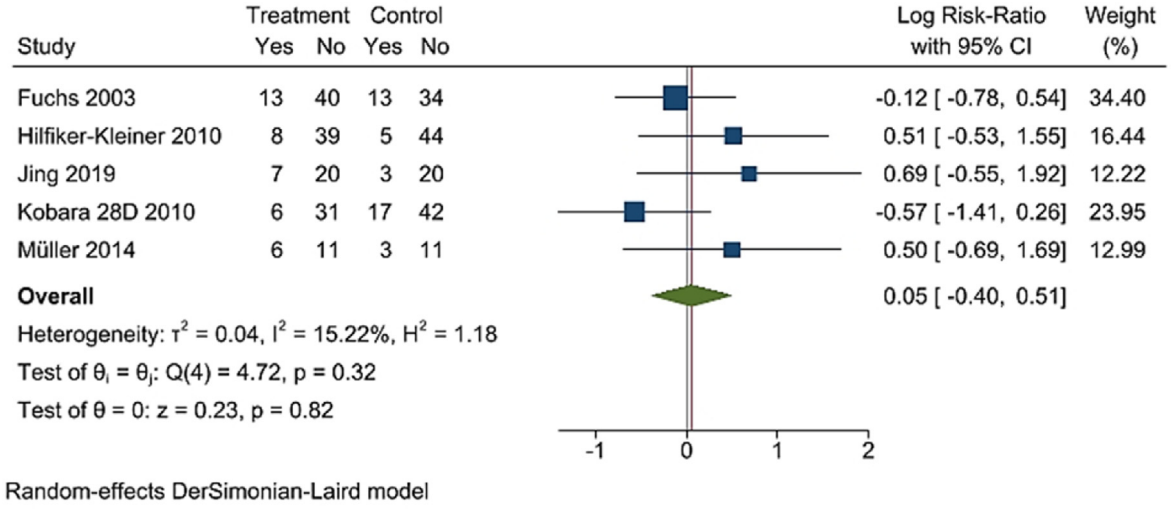


Figure 5. Quantitative analysis of studies evaluating the effects of IL-6 inhibition on the mortality rate: Forest Plot of studies investigating mortality rate using random-effect model (logs Risk ratio, 95% CI). The plot shows pooled and individual risk ratio of each study.

coefficient and the p-value suggest the change is significant (Table 5.). The bubble plot reveals elevated infarct size with increase in the duration of ischemia.

4. Discussion

4.1. Summary of the current study

To our knowledge, this is the first systematic review and meta-analysis of pre-clinical studies on IL-6 in animal models of myocardial infarction to assess the effects of IL-6 on post-MI cardiac function and morphology. These results, as opposed to the widely believed notion, suggest a minor role of IL-6 in the heart, cardiomyocyte survival as well as in the cardiac remodeling post-MI, specifically in cardiac mortality, infarct size, ejection fraction and immune cell infiltration. Blocking the receptor IL-6R with anti-IL-6R antibody had shown substantial improvement against inflammatory pathology in humans, however inhibiting IL-6 with anti-IL-6 antibody deteriorated the survival of the animals [18, 23]. In addition, systemic loss of IL-6 reported no significant effects on the survival post-MI [16, 25]. The results reported an increase in fraction shortening in pre-clinical settings using IL-6 inhibitors under ischemic conditions. The lack of IL-6 showed no effect on LVEDD and a slight increase in the LVESD. Additionally, the rate of contraction and relaxation remained unchanged post-infarction. The migration of the neutrophils was reduced to the infarct site post-infarction in both pre-clinical and clinical studies. In clinical trials, inhibition of IL-6R with TCZ reduced CRP and Tnt levels in MI patients.

4.2. Effects of IL-6 inhibition on cardiac remodeling and function

In all the included studies, suppressing IL-6 signaling imposed no significant effects on infarct size. Subgroup analysis shows no between group differences on change in infarct size in both genetic and antibody mediated inhibition of IL-6. Interestingly in a study, Wistar rats when treated with a complex of IL-6 and sIL-6R showed reduced cardiomyocyte

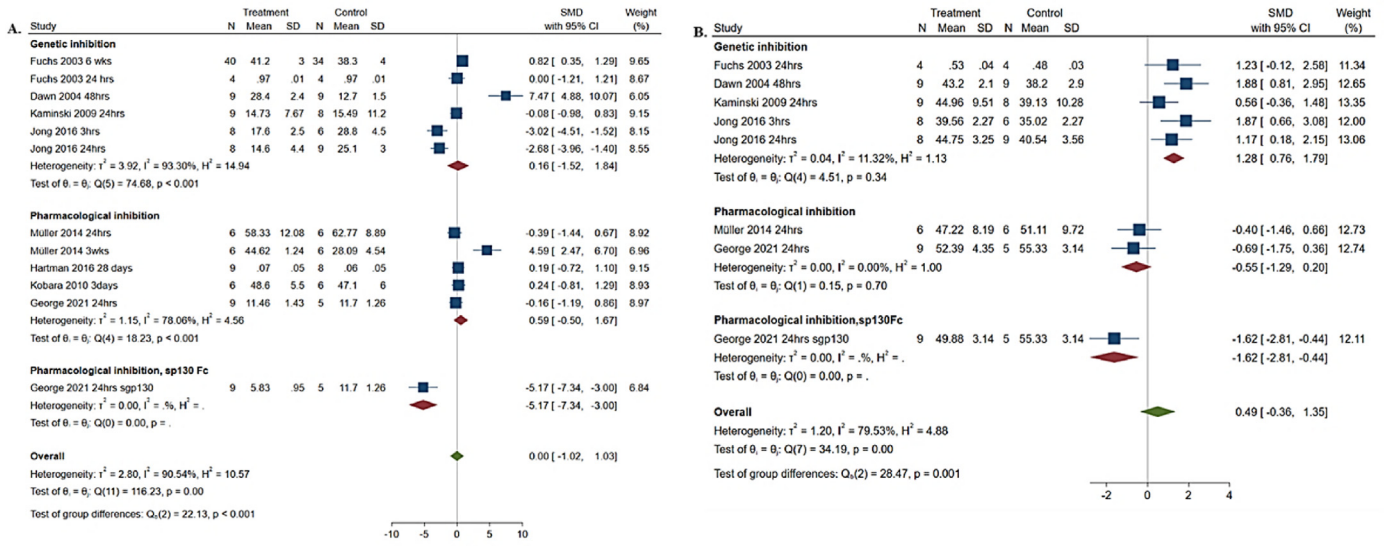


Figure 6. Quantitative analysis of studies estimating the effects of IL-6 inhibition on infarct size and area at risk: Forest plot of studies measuring (A.) infarct size and (B.) Forest plot of studies assessing the area at risk using random-effect model (SMD, 95% CI). The plot shows individual and pooled SMD along with the relative weight of each study.

apoptosis and mitigated the infarct size [37]. Anti-IL-6 treated mice reported an evident scar size post three weeks of acute myocardial ischemia. Similarly anti-IL-6 treated rats failed to reduce infarct size and AAR post I/R. Conversely, trans-signaling blockade of IL-6 pathway via fusion protein sgp130Fc attenuated neutrophil infiltration and significantly reduced the infarct size post MI [26]. Due to varying results obtained from all the included studies, the change in the infarct size remains insignificant. The overall effect size from the included study suggests a significant increase in the AAR region. However, sub-grouping of studies reveals genetically knockout murine model with increased AAR and contrastingly pharmacological inhibition of IL-6 lead to reduced AAR. This shows systemic inhibition of IL-6 has more deleterious effect on myocardium than restricted inhibition of IL-6 in animal model.

We also observed a significant reduction in both fibrosis and cardiomyocyte cross-sectional area. Single-dose of MR16-1 (IL-6R Ab) significantly reduced fibrosis and cardiomyocyte cell size [23]. But the periodic dosage of MR16-1 (2mg/mouse for four weeks) showed no significant changes in both fibrosis and cross-sectional area of cells [22]. Likewise hypertensive and diabetic IL-6 KO mice models elicited a decrease in fibrosis and inflammation [38, 39]. Meta-analysis of the selected studies and these results indicate a minimal to no role of IL-6 inhibition on cardiac remodeling. A recent stated that blocking the trans-signaling of IL-6 has more beneficial and cardio-protective functions, studies exploring the effects of IL-6R inhibition could further generate a potential understanding of cardiac remodeling in these animals [26].

Inhibition of functional IL-6 enhanced cardiac functions by improving the fraction shortening. Although the change in the ejection fraction was significantly reduced and reported high heterogeneity suggesting mixed results from different studies. Mice treated with anti-IL-6 Ab, before and after ischemic-reperfusion showed reduced ejection fraction [18]. Genetic deletion of IL-6 in neonatal mice displayed a reduction in cardiac function as both ejection fraction and fraction shortening decreased [17]. Whereas, studies targeting IL-6R via antibody or tissue-specific gp130 KO showed better cardiac function by improving the ejection fraction and fraction shortening [23, 25]. Inhibition of trans-signaling pathway of IL-6 using sgp130Fc showed improvement of cardiac function [26].

4.3. IL-6 and immune cell infiltration

Earlier studies on IL-6 have proposed that an upsurge in the IL-6 levels post-ischemia increases the intercellular adhesion molecule (ICAM) expression on cardiomyocytes which further induces the migration of immune cells at the ischemic site [40]. This process is very dynamic and dependent on the duration of ischemia. Restricting the functional IL-6 via anti-IL-6 antibody strongly inhibited the accumulation of neutrophils and macrophages at the infarct site 72 h post-myocardial infarction [18]. In the ischemia-reperfusion on the IL-6 KO mouse, the influx of neutrophils after 30 min and 3 h of reperfusion was undetectable. However, post 24 h reperfusion a significant influx was reported in both wildtype and IL-6 KO [19]. In the case of tissue-specific gp130 KO mice, there was a reduction in inflammation due to limited migration of leukocytes and macrophages to the infarct site 2 weeks post-infarction. Moreover, similar results were reported in NSTEMI patients treated with TCZ leading to the reduced number of infiltrated neutrophils, leukocytes and platelets [27, 32]. All these studies illustrate a vital role of IL-6 in the migration of immune cells at infarct sites. In addition to its role as a

Table 4. The effect size of secondary outcomes included in the review.

Outcome/Subgroup	No. of studies	No. of animals	Effect Estimate	p value	Heterogeneity, I^2 (p value)
Fibrosis	4	97	-0.17 [-1.47, 1.14]	0.80	87.81% ($p < 0.00$)
Cardiomyocyte CSA (mm)	4	93	-1.10 [-2.24, 0.03]	0.06	81.80% ($p < 0.00$)
Heart rate	4	113	-0.69 [-1.42,0.03]	0.06	70.56% ($p = 0.001$)
IL-6 levels	6	149	5.66 [3.25, -8.06]	0.001	93.46% ($p = 0.00$)
dP/dT max (mmHg/sec)	3	35	-0.61 [-1.76, 0.53]	0.29	66.99% ($p = 0.05$)
dP/dt min (mmHg/sec)	3	35	0.84 [0.19, 1.50]	0.01	2.24% ($p = 0.36$)

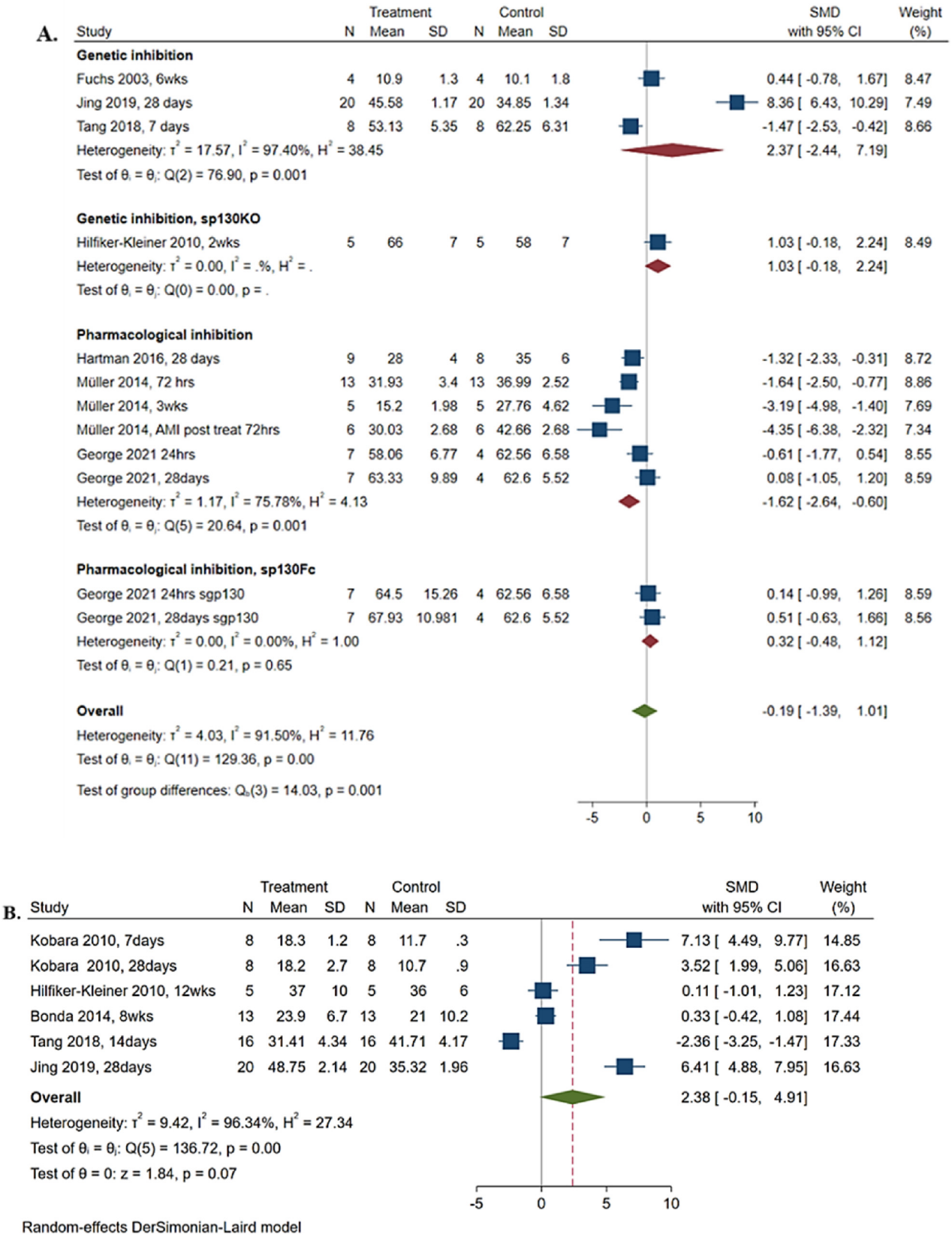


Figure 7. Quantitative analysis of studies determining the overall effect of IL-6 inhibition on cardiac function (A.) Forest plots of studies estimating ejection fraction and (B.) Forest plot of studies estimating fraction shortening using random-effect model (SMD, 95% CI). The plot shows combined and individual SMD along with the relative weight of each study.

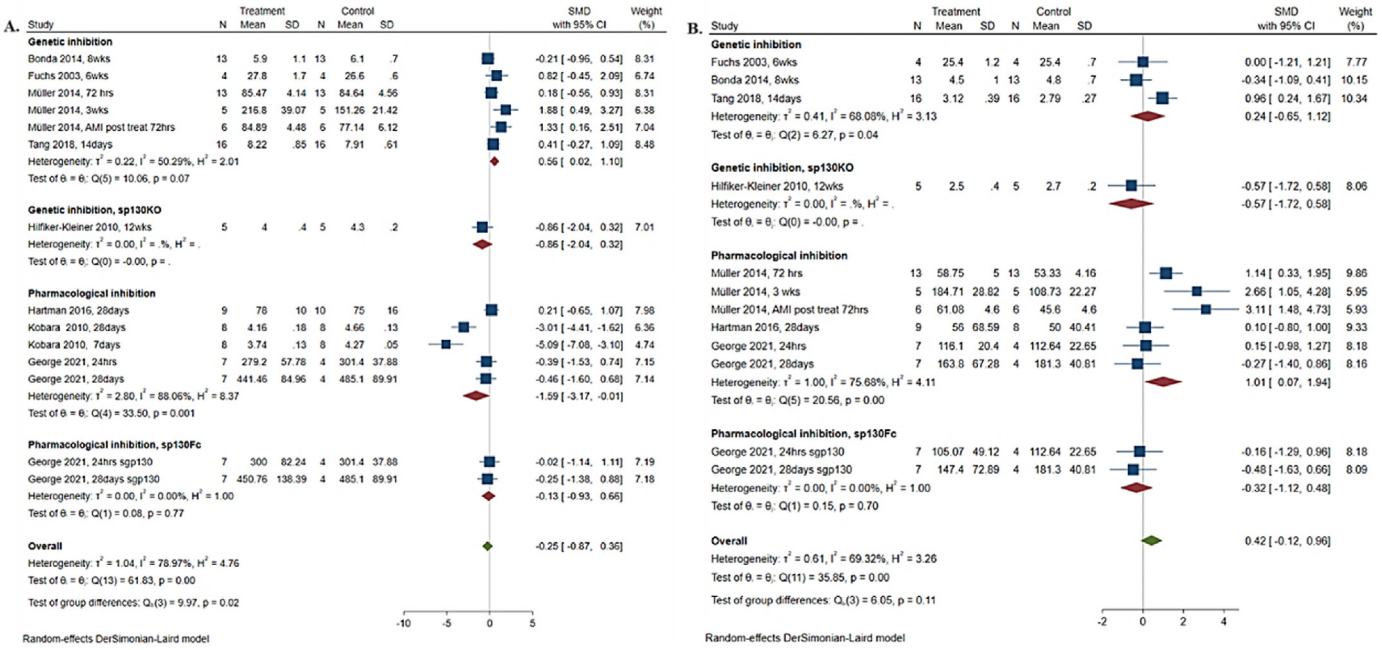


Figure 8. Quantitative analysis of studies determining the overall effect of IL-6 inhibition on left ventricular diastolic and systolic diameter: Forest plot of studies measuring (A) LVEDD in millimeter (LV-end diastolic Diameter) and relative volume units (LV- diastolic volume) and (B) LVESD in millimeter (LV-end diastolic Diameter) and relative volume units (LV- diastolic volume) using the random-effect model and relative weight of respective studies.

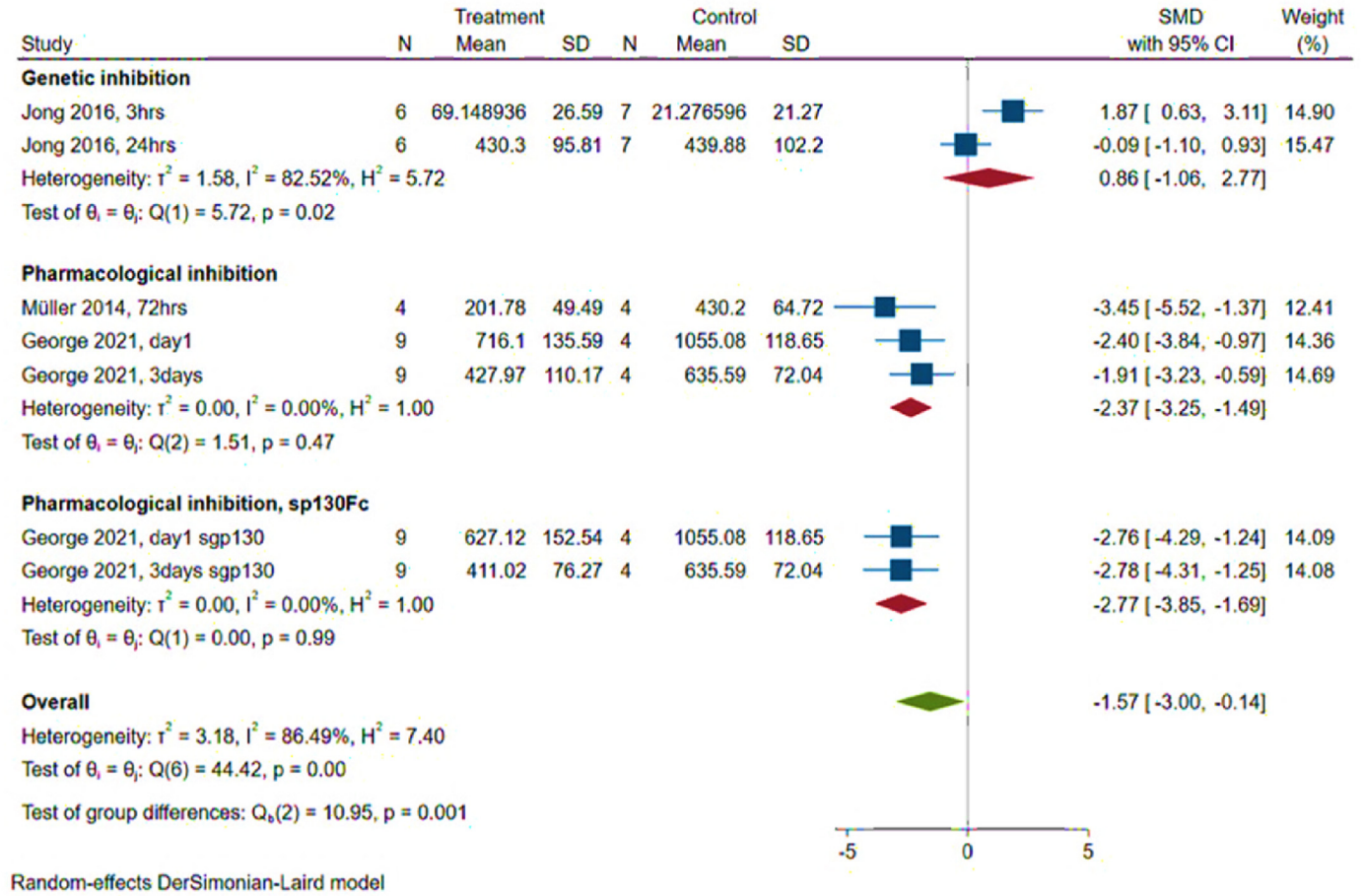
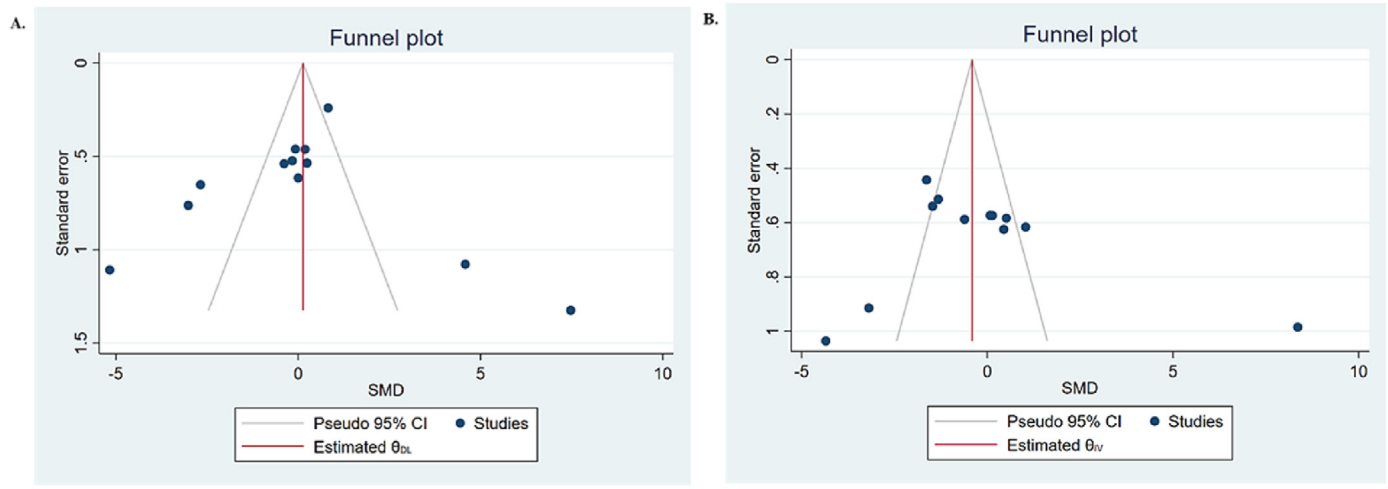


Figure 9. Quantitative analysis of studies estimating the effect of IL-6 inhibition on neutrophil cell count: Forest of plot studies measuring neutrophil cells in control and ischemic murine model using study random effect model (SMD, 95% CI). The diamond in the plot indicates the overall SMD change and heterogeneity values.



Publication Bias Assessment for infarct size

Test Name	value	p
Fail-Safe N	0.000	0.319
Begg and Mazumdar Rank Correlation	-0.121	0.638
Egger's Regression	1.363	0.173
Trim and Fill Number of Studies	0.000	.

Publication Bias Assessment for ejection fraction

Test Name	value	p
Fail-Safe N	0.000	0.062
Begg and Mazumdar Rank Correlation	0.212	0.381
Egger's Regression	0.644	0.520
Trim and Fill Number of Studies	0.000	.

Figure 10. Publication bias assessment: Funnel plot of included studies (A.) Egger's regression of infarct size and (B.) Egger's regression of Ejection fraction.

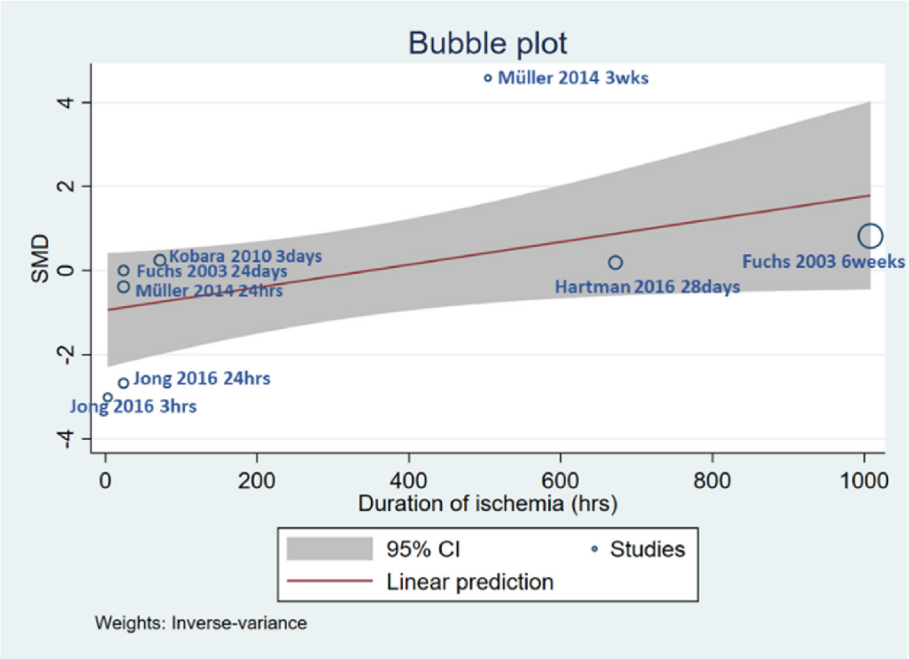


Figure 11. Meta-regression of included studies: Meta-regression of infarct area to duration of ischemia (hrs.) post-surgery.

pro-inflammatory cytokine, studies also mentioned the obligatory role of IL-6 in cardio protection by activating the JAK-STAT pathway [21, 41]. In the post-ischemic murine model, Muller et al. 2014 reported that endogenous IL-6 transiently increases the hyaluronan-matrix which enhances the process of wound healing by promoting the myofibroblastic phenotype of cardiac fibroblasts [18]. Hence, soon after myocardial infarction, IL-6 acts as a pro-inflammatory signal and induces the migration of immune cells to the infarct site, and in the later stages of ischemic injury, IL-6 takes part in the activation of the cardioprotective pathways [42].

4.4. Genetic vs pharmacologically inhibited IL-6 model

To date, genetically inhibited IL-6 mice models are used to explore the role of IL-6 under ischemic stress. However, genetically inhibited IL-6

Table 5. Meta-regression analysis of infarct size to duration of ischemia.

Meta estimate	coefficient	Std. Error	z	p> |z|	95% CI
Duration of ischemia (hrs)	0.0027103	0.0014291	1.90	0.058	0.005, 0.4091

mice subjected to MI failed to produce consistent results in different studies. IL-6 KO in neonatal mice resulted in reduced cardiomyocyte proliferation and cardiac function [17]. In prolonged myocardial ischemia IL-6 KO failed to generate significant effects on mortality, infarct size and cardiac function. The meta-regression analysis revealed that the infarct size tends to increase with the duration of ischemia in both pharmacologically inhibited IL-6 and genetically knocked out murine models. Moreover, pharmacological inhibition of IL-6 with anti-IL-6 antibody led to increased infarct size and reduced ejection fraction. In contrast to IL-6 inhibition, blocking the IL-6R with MR16-1 post-ischemia suppressed inflammation in the myocardium which further enhanced the LV remodeling. Likewise, IL-6 trans-signaling inhibition via recombinant fusion protein sgp130Fc in rats resulted in reduced infarct size, inflammatory cell migration and improved ejection fraction post 50min I/R. In addition, they also treated rats with anti-IL-6 antibody for the blockade of the classic signaling which failed to improve the cardiac function and remodeling. Therefore, the authors suggest blocking trans-signaling has greater anti-inflammatory effects and preserving cardiac function compared to blocking the classic signaling in MI [26]. Moreover, we cannot ignore the fact that the involvement of IL-6 post-infarction is very dynamic and limited studies have investigated the cardioprotective role in ischemic tissue. Additional studies are required exploring the role of IL-6 in protecting the cardiomyocytes post-infarction. As described previously IL-6 contributes not only in pro- but also in anti-inflammatory and anti-apoptotic pathways [43, 44, 45], there is a high possibility that studies did not observe any significant effects on cardiac remodeling and function post-ischemia. These results also suggest functional inhibition of the IL-6 molecule is compensated by other cytokines in the milieu, however targeting common receptor, does not allow other cytokines to compensate the functions. Yet more studies focusing on the effects of IL-6R inhibition in cardiac remodeling are required to understand the pro-inflammatory role of IL-6 and this model will have more clinical relevance compared to genetic deletion of IL-6. In addition, we failed to find any published studies exploring the role of IL-6 inhibition post MI in more species like rabbit, pig and other higher animals.

4.5. Serum levels of IL-6

Several experimental and clinical studies reported a significant increase in the serum IL-6 levels post-myocardial ischemia [46, 47]. In addition, an upsurge in the IL-6 levels is also stated in the infarct areas post-ischemia. Increased serum levels of IL-6, IL-6R and CRP are used as biomarkers to report acute myocardial infarction in patients [48]. Interestingly studies also reported an increase in IL-6 levels in patients treated with TCZ [27]. Blocking the IL-6R causes a decline in IL-6 clearance from the circulation and thus results in higher serum IL-6 levels.

4.6. Heterogeneity

In our meta-analysis, we observed a high degree of heterogeneity in primary endpoints between the studies. Though all the experimental models appear to be homogenous, in age and surgical procedure used, still they showed different study characteristics. The heterogeneity of the studies can be explained by the differing myocardial infarction duration, dosage of antibodies and variability in endpoints measurements. In the evaluation of the risk of bias, all studies failed to report allocation concealment, reproducibility testing, design and execution. As described by Hoojimans et al. 2014 [14] there is no standard protocol for randomization in animal experiments; so only a few studies follow the random sequence generation and allocation concealment. Similarly, in our included studies we observed a high bias, however, few recent publications reported blinding of outcome assessment, random sequence generation.

4.7. Clinical trials

So far very few studies have been conducted to investigate the effects of IL-6 inhibition on cardiac function in MI patients. In our review, we have mentioned the characteristics of the included studies in detail (dosage, duration and adverse events). Two clinical trials with differential drug dosage, administration route and time duration resulted in discrepancy reports. The trial with 280mg TCZ treated patients showed a significant reduction in high sensitivity CRP levels and TnT levels in the initial days, while the trial with 162mg TCZ failed to lower CRP levels [27, 28]. These data suggest the dose and time-dependent effects of IL-6R antibody in MI patients. Borch et al.2021 reported improved myocardial salvage index in TCZ treated group compared to the placebo. Additionally they also stated less extensive microvascular obstruction however, failed to showed significant reduction in infarct size in the TCZ group [33].

Although, several studies revealed inhibiting IL-6 in rheumatoid arthritis patients reduced the cardiovascular risk and improved endothelial function [49, 50], yet tocilizumab failed to improve coronary endothelial function in NSTEMI patients [32]. Though a few Mendelian randomization studies speculated blocking IL-6R is associated with reducing CAD, more randomized trials are required to replicate the findings [51, 52]. Currently there are no active trials inspecting the effects of IL-6 inhibitors on MI patients. Studies exploring the inhibitory effects of IL-6 in bigger population size will provide a better understanding of the involvement of IL-6 in cardiac remodeling.

4.8. Limitations

The current meta-analysis is limited primarily due to the small number of conducted pre-clinical and clinical studies. Inconsistent data obtained from available knock-out mice studies weakens the implication of IL-6 in mortality, cardiac function and remodeling. There are limited pharmacological inhibition studies to report a conclusive effect of IL-6 in cardiac remodeling. Controversial data from population studies treated with anti-IL-6R antibody, questions the causative role of IL-6 post-infarction.

5. Conclusion

We conclude from our meta-analysis that inhibiting IL-6 does not implicate a significant change in cardiac remodeling post-myocardial ischemia, but needs further studies exploring the effects of IL-6R inhibition in animals. So far, limited clinical trials are investigating the effects of IL-6R inhibitors in MI patients. Added population studies will support the contribution of IL-6 in cardiac function and inflammation biology.

Declarations

Author contribution statement

Sushmitha Duddu: Performed the experiments, analyzed and interpreted the data, wrote the paper.

Mohan Agrawal: Analyzed and interpreted the data, wrote the paper.

Rituparna Chakrabarti, Ashutosh Tiwari and Praphulla Chandra Shukla: Conceived and designed the experiments, analyzed and interpreted the data, wrote the paper.

Anuran Ghosh: Conceived and designed the experiments, analyzed and interpreted the data.

Nishant Chakravorty: Conceived and designed the experiments, wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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