ORIGINAL RESEARCH ARTICLE

Effect of immunomodulation on cardiac remodelling and outcomes in heart failure: a quantitative synthesis of the literature

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Abstract

Aims   Immunomodulation in heart failure (HF) has been studied in several randomized controlled trials (RCTs) with variable effects on cardiac structure, function, and outcomes. We sought to determine the effect of immunomodulation on left ventricular ejection fraction (LVEF), LV end-diastolic dimension (LVEDD), and all-cause mortality in patients with HF with reduced ejection fraction (HFrEF) through meta-analyses and trial sequential analyses (TSAs) of RCTs.

Methods and results   PubMed, Embase®, Cochrane CENTRAL, and ClinicalTrials.gov were systematically reviewed to identify RCTs that studied the effects of immunomodulation in patients with HFrEF. The primary endpoint in this analysis was change in LVEF. Secondary outcomes were changes in LVEDD and all-cause mortality. TSA was used to quantify the statistical reliability of data in the cumulative meta-analyses. Nineteen RCTs with 1341 HFrEF subjects were eligible for analyses. The aetiology of HF, specific immunomodulation strategy, and treatment duration were variable across trials. Immunomodulation led to a greater improvement in LVEF [mean difference: +5.7% 95% confidence interval (CI): 3.0–8.5%, $P < 0.001$] and reduction in LVEDD (mean difference: -3.7 mm, 95% CI: -7.0 to -0.4 mm, $P = 0.028$) than no immunomodulation in meta-analyses and TSAs. We observed a non-significant decrease in all-cause mortality among those on immumomodulation (risk ratio: 0.7, 95% CI: 0.4–1.3, $P = 0.234$), but the Z-curve for cumulative treatment effect of immunomodulation in the TSA did not cross the boundary of futility.

Conclusions   Immunomodulation led to improved cardiac structure and function in patients with HFrEF. While these benefits did not translate into a significant improvement in mortality, our analysis suggests that larger studies of targeted immunomodulation are needed to understand the true benefits.

Introduction

Heart failure (HF) is a pro-inflammatory state.[1,2] Several pathways including pro-inflammatory cytokines and innate and adaptive immune cellular responses have been implicated in HF.[3–7] It is also evident that dysregulated inflammation and immune activation contribute importantly to progressive left ventricular (LV) remodelling and dysfunction.[4,8–10] Hence, inflammatory and immune pathways are theoretically appealing as therapeutic targets in HF to improve adverse LV remodelling and prognosis. Several, generally small, randomized controlled trials (RCTs) have evaluated a variety of immunomodulation strategies in patients with HF with variable degrees of success.[4,9,11] These approaches have ranged

from broad immunomodulation targeting multiple pathways to drugs and/or biologics targeting specific cytokines or cellular components of inflammation. Anti-cytokine approaches in HF to inhibit tumour necrosis factor (TNF)-α[1] and interleukin (IL)-1[11] did not yield appreciable benefits in mortality or major adverse cardiovascular events, although a recent sub-analysis of the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) suggested that canakinumab, an IL-1β monoclonal antibody, imparted a dose-dependent reduction in HF-related mortality and hospitalizations in individuals with previous myocardial infarction and increased high-sensitivity C-reactive protein.[12]

These more recent data, as well as rapid advances in our understanding of the basis for inflammation and immune activation in HF,[5,13] have underscored the need for a reappraisal of therapeutic immunomodulation in this disease.[14] Nonetheless, robust data from large-scale clinical trials regarding the combined effects of immunomodulation on cardiac remodelling and clinical outcomes in HF are few. Accordingly, we conducted a systematic review and meta-analyses to evaluate the aggregate benefit of immunomodulation on LV structure and function and all-cause mortality in HF with reduced ejection fraction (HFrEF) in trials that reported both outcomes. To ascertain the robustness of the treatment signals, we also employed trial sequential analysis (TSA) to improve the precision of effect sizes over time, as data have continued to accumulate in the field of immunocardiology and HF.

Methods

Protocol and registration

This study was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (*Table S1*). The review was registered with PROSPERO (International Prospective Register of Systematic Reviews; registration number CRD42019138909). Because this was a meta-analysis and systematic review of trial results that have already been published, approval of institutional review board and ethics committee was not required.

Eligibility criteria

RCTs enrolling patients with HFrEF randomized to receive either immunomodulation or placebo and reporting change in LV ejection fraction (LVEF) were included in the analyses (*Data S1*). The search strategy and data collection are detailed in the Supporting Information (*Data S2*).

Risk of bias in individual studies

Risk of bias (RoB) was assessed using the Cochrane RoB tool modified to capture the components of random sequence generation; allocation concealment; blinding of participants; blinding of outcome assessment; and analysis of incomplete outcome data.

Outcomes

The primary endpoint in this analysis was change in LVEF in patients with HF receiving immunomodulation as compared with no immunomodulation. Secondary outcomes were the change in LV end-diastolic dimension (LVEDD) and all-cause mortality.

Meta-analysis and publication bias

Random effects modelling was used to estimate pooled mean difference for continuous outcomes (change in LVEF and LVEDD; pretreatment and post-treatment) and risk ratio (RR) for the categorical outcome (all-cause mortality). The details for statistical analysis are presented in S4 Statistical section.

Trial sequential analysis

TSA was used to quantify the statistical reliability of data in the cumulative meta-analyses by adjusting significance levels for sparse data and repetitive testing on accumulating data. A cumulative *Z*-curve was plotted against the accrued sample size. Sequential boundaries for benefit, futility, and required information size for a conclusive meta-analyses were constructed, assuming the cumulative treatment effect for each outcome, α = 0.05, and β = 0.20. The details for statistical analysis are presented in *Data S3*. All analyses were performed using STATA V15.0 (College Station, TX, USA) statistical software and TSA software version 0.9.5.10 Beta.

Sensitivity and subgroup analyses

The effect of exclusion of studies with a high RoB on the primary outcome was estimated. The effect of immunomodulation on outcomes that showed a signal for benefit was also studied in the subgroup analyses by drug dividing into two subgroups by type of immunomodulation, that is, broad immunosuppression vs. anti-cytokine immunotherapy, as well as by classifying studies into three groups on the basis of the aetiology of HF: ischaemic, non-ischaemic, and mixed in each study.[1] With regard to the type of

Table 1 Treatment protocol and baseline characteristics in the included trials

S.no	Author/year	Treatment protocol	Mechanism of immunomodulation	Assessment of outcome	Total (n)	Treatment arm (n)	Control arm (n)	Mean age (years)	Women (%)	NICM (%)	LVEF (%)	LVEDD (mm)	ACEi–ARBs/beta-blockers/Aldosterone antagonist/digoxin (%)
1	Parrillo[17]/1989	Prednisone	Broad	3 months	101	49	52	43	NR	100	17.5	68.7	NA/NA/NA/NA
2	Sliwa[18]/1998	Pentoxifylline	Anti-cytokine	6 months	28	14	14	52	32.1	100	24.2	64.5	100/NA/NA/100
3	Deswal[19]/1999	Etanercept	Anti-cytokine	2 weeks	18	12	6	63.3	5.6	83.3	27.5	NR	100/11/NA/94.4
4	McNamara[20]/2001	IVIg	Broad	1 year	62	33	29	43	4	100	25	NR	90/18/NA/NA
5	Skudicky[21]/2001	Pentoxifylline	Anti-cytokine	6 months	39	19	20	48.5	33.3	100	24	68.5	100/100/NA/100
6	Bozkurt[22]/2001	Etanercept	Anti-cytokine	3 months	47	31	16	55	19	36.2	18.4	NR	100/47/NA/87
7	Gullestad[23]/2001	IVIg	Broad	26 weeks	39	19	20	60.5	17.5	57.5	27	NR	100/75/NA/40
8	Wojnicz[24]/2001	Prednisone + azathioprine	Broad	3 months	84	41	43	40	17.9	100	24.4	67.1	100/100/NA/100
9	Sliwa[25]/2002	Pentoxifylline	Anti-cytokine	1 months	15	8	7	46	16.7	100	15.4	69.1	100/NA/NA/100
10	Chung[26]/2003	Infliximab	Anti-cytokine	14 weeks	150	101	49	61.3	19	35.3	24	NR	100/73/39/78
11	Bahrmann[27]/2004	Pentoxifylline	Anti-cytokine	6 months	41	21	20	56.5	6.4	57.4	28	69	100/96/NA/51
12	Sliwa[28]/2004	Pentoxifylline	Anti-cytokine	6 months	33	19	14	55.1	28.9	0	25	61.2	100/100/50/NA
13	Torre-Amione[29]/2005	Celecade[a]	Broad	6 months	74	37	37	61.7	31.1	51.4	22.2	NR	89/51/46/82
14	Gullestad[30]/2005	Thalidomide	Anti-cytokine	12 weeks	48	22	26	66	25	32.2	24.6	NR	99/91/NA/29
15	Gong[31]/2006	Methotrexate	Broad	12 weeks	62	30	32	62.4	48.1	59.7	31	62.7	94/45/NA/68
16	Frustaci[32]/2009	Prednisone + azathioprine	Broad	6 months	85	43	42	42.8	40	100	27.1	68.6	100/100/NA/100
17	Deftereos[33]/2014	Colchicine	Broad	6 months	267	134	133	66.7	33	28	27.6	61.7	85/79/62/NA
18	Van Tassell[34]/2016	Anakinra	Anti-cytokine	12 weeks	52	34	18	57.7	27	65.4	31.2	NR	83/92/52/NA
19	Xiaojing[35]/2017	Thymopentin	Broad	75 days	96	48	48	70.3	43.8	20.8	35.5	61.8	81/77/92/43

EF, ejection fraction; IVIg, intra-venous immunoglobulin; LVEDD, left ventricular end-diastolic dimension; LVEF: left ventricular ejection fraction; NA, not available; NICM, non-ischaemic cardiomyopathy; NR, not recorded.
[a]Autologous blood transfusion.

immunomodulation, in addition to direct anti-cytokine neutralization strategies (e.g. etanercept, infliximab, and anakinra), both pentoxifylline and thalidomide were considered as anti-cytokine approaches given the known effects of these drugs in reducing TNF expression.[4] All other treatments were considered as broad immunomodulation strategies. Three important trials,[15,16] which were not included based on our pre-specified inclusion criteria (change in LVEF not available), reported all-cause mortality in patients with HFrEF receiving immunomodulation. We also performed a sensitivity analysis for mortality after including these three trials to estimate any change in the treatment effect estimate for mortality.

Results

### Baseline and treatment characteristics

Of the 685 records screened, 19 studies with 1,341 patients met our inclusion criteria (*Figure S1*). *Table 1* highlights the baseline characteristics of patients. The mean age of the trial population ranged from 43 to 70 years. The sample size in the RCTs varied from 15 to 267. Mean LVEF was 23.8% [95% confidence interval (CI): 22.0–25.6%]. There were 11 studies with both ischaemic (ICM) and non-ischaemic cardiomyopathy (NICM): one study with only ICM and seven studies with only NICM. The percentage of patients with NICM in studies with both ICM and NICM varied from 20.8% to 83.3%. Broad immunosuppression was used in nine studies (64.9% patients),

and anti-cytokine immunosuppression was used in 10 studies (35.1% patients). Treatment duration varied from 2 weeks to 6 months. The treatment regimens of the included studies are given in *Table S2*. The RoB was deemed to be acceptable (*Figures S2 and S3*) for all except one study.[35] Echocardiography was used for LVEF assessment in 10 studies (61.6% patients), while radionuclide imaging was used in nine studies (38.4% patients) (*Table S2*).
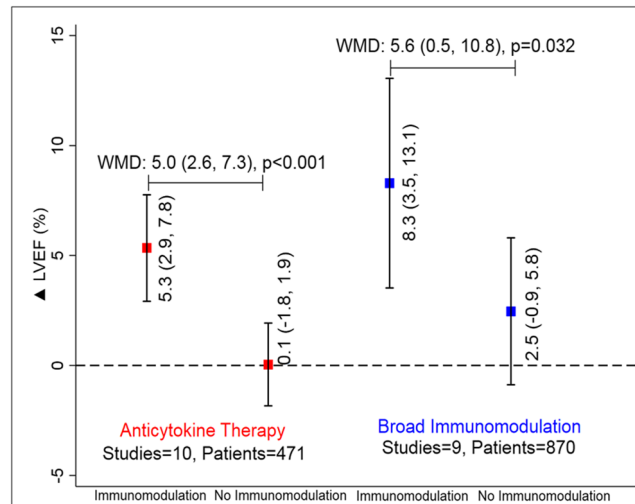
Effect of immunomodulation on left ventricular ejection fraction

The mean difference in change in LVEF was +5.7% (95% CI: +3.0 to 8.5%, $P < 0.001$) (*Figure 1A* and *Figure S4*) higher with immunomodulation as compared with no immunomodulation. There was no evidence of publication bias ($P > 0.05$ for both Egger and Begg test for small-study effects) (*Figure S5*) for this estimate. The effect estimate for improvement in LVEF remained similar (5.5%) to overall estimate (5.7%) after removing the study with high RoB.[35] There was directional consistency in LVEF improvement upon immunomodulation with only one study[27] reporting non-significant improvement in LVEF among those not on immunomodulation (*Figure S4*). LVEF improvement was consistently higher when studies were stratified by type of immunomodulation; 5.0% (95% CI: 2.6–7.3, $P < 0.001$) with anti-cytokine therapy and 5.6% (95% CI: 0.5–10.8, $P < 0.001$) with broad immunomodulation (*Figure 2* and *Figure S4*). The improvement in LVEF was also seen across

Figure 1 Difference in change in left ventricular ejection fraction (LVEF) (A), left ventricular end-diastolic dimension (LVEDD) (B), and mortality (C) between immunomodulation and no immunomodulation. WMD, weighted mean difference. Data are presented as mean (95% confidence interval). The black square represents the mean change in parameters with or without immunomodulation with the error bars representing the 95% confidence interval.
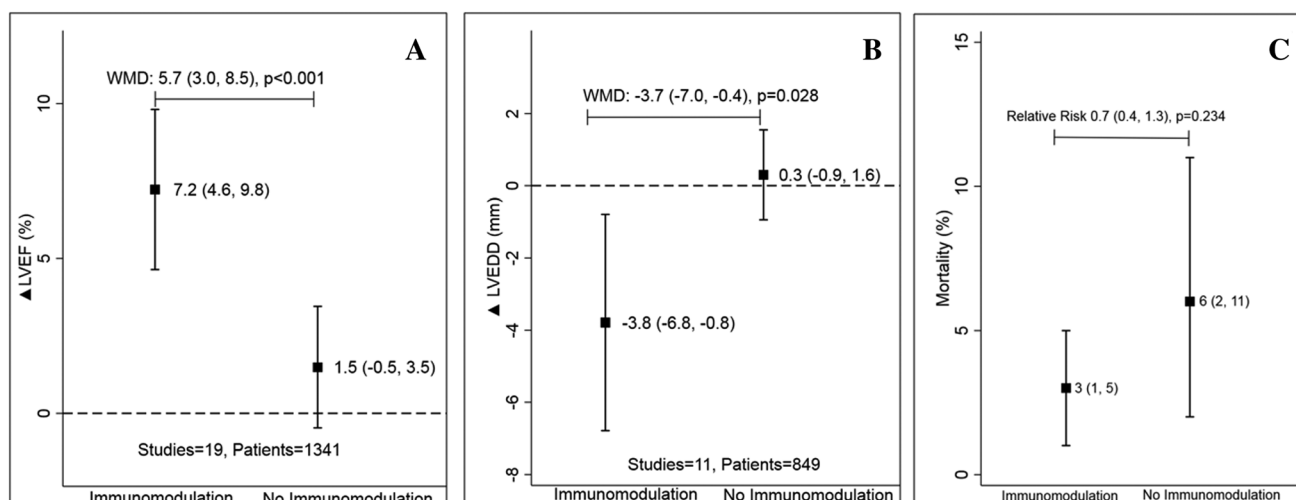
Figure 2 Improvement in left ventricular ejection fraction (LVEF) with or without immunomodulation with anti-cytokine therapy (red) and broad immunomodulation (blue). WMD, weighted mean difference. Data are presented as mean (95% confidence interval). The solid square represents an improvement in LVEF with or without immunomodulation with the error bars representing the 95% confidence interval.

Figure 3 Improvement in left ventricular ejection fraction (LVEF) with or without immunomodulation in studies with non-ischaemic (red), ischaemic (blue), and mixed non-ischaemic and ischaemic aetiology (green) of heart failure. WMD, weighted mean difference. Data are presented as mean (95% confidence interval). The solid square represents an improvement in parameters with or without immunomodulation with the error bars representing the 95% confidence interval.
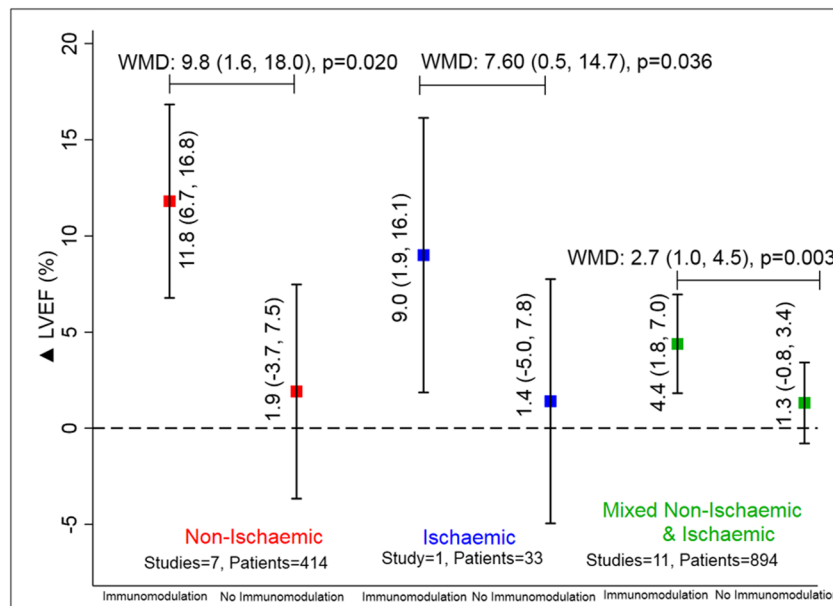
studies enrolling patients with ischaemic, non-ischaemic, or mixed aetiology patients: 9.8%, 7.6%, and 2.7%, respectively (all $P < 0.05$) (*Figure 3* and *Figure S6*). The weighted mean difference (WMD) and the standardized mean difference (SMD) for improvement in LVEF are presented in *Table S3* and were consistent in magnitude and direction.
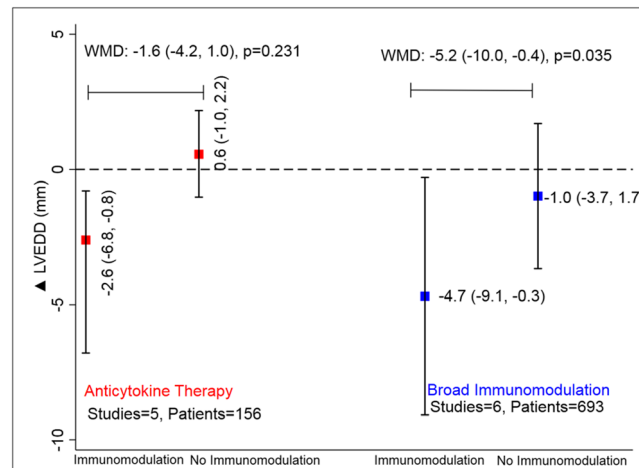
Effect of immunomodulation on left ventricular end-diastolic dimension

Eleven studies reported a change in LVEDD (*n* = 849). Of them, five (18.3% patients) used anti-cytokine immunotherapy and six (81.7% patients) used broad

immunomodulation (*Table 1*). The mean change in LVEDD was 3.7 mm lower (95% CI: −7.0 to −0.4, *P* = 0.028) with immunomodulation as compared with no immunomodulation (*Figure 1B*). The improvement varied from −19.2 to 0.0 mm (*Figure S7*). The LVEDD was numerically lower with both with anti-cytokine immunotherapy (−1.6 mm, 95% CI: −4.2 to 1.0, *P* = 0.231) and broad immunomodulation (−5.2 mm, 95% CI: −10.0 to −0.4, *P*

= 0.035) (*Figure 4*). This improvement in LVEDD was seen across studies enrolling patients with non-ischaemic or mixed aetiology patients, −5.2 and −3.7 mm, respectively (*Figure 5* and *Figure S8*), but did not reach statistical significance individually in either group individually. The WMD and SMD for improvement in LVEDD for the overall meta-analysis are presented in *Table S3* and were consistent in magnitude and direction.

Figure 4 Improvement in left ventricular end-diastolic dimension (LVEDD) with or without immunomodulation with anti-cytokine therapy (red) and broad immunomodulation (blue). WMD, weighted mean difference. Data are presented as mean (95% confidence interval). The solid square represents the mean change in parameters with or without immunomodulation with the error bars representing the 95% confidence interval.

Figure 5 Improvement in left ventricular end-diastolic dimension (LVEDD) with or without immunomodulation in studies with non-ischaemic (red), ischaemic (blue), and mixed non-ischaemic and ischaemic aetiology (green) of heart failure. WMD, weighted mean difference. Data are presented as mean (95% confidence interval). The solid square represents the mean change in parameters with or without immunomodulation with the error bars representing the 95% confidence interval.
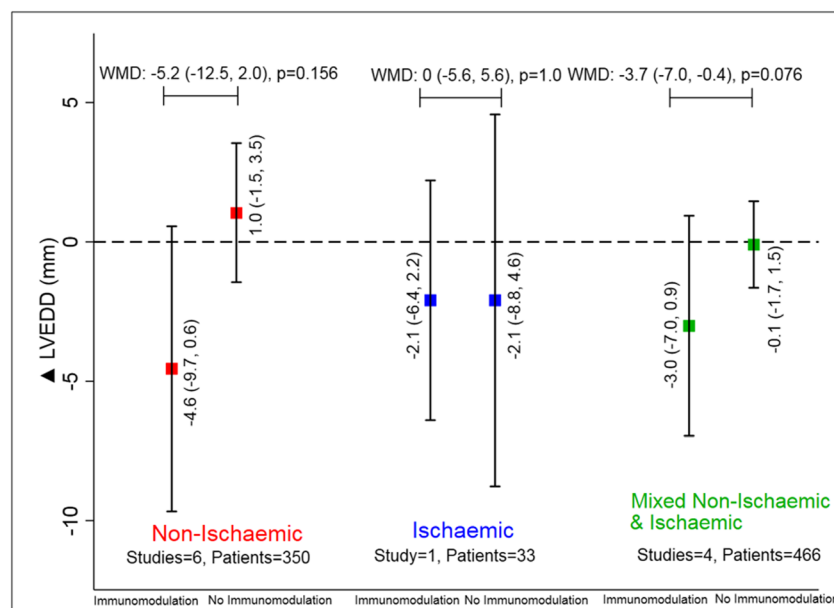
Effect of immunomodulation on mortality

In 14 studies (n = 1099) reporting mortality, the risk of mortality was 2.7% vs. 6.0% (RR 0.7, 95% CI: 0.4–1.3, P = 0.234) with immunomodulation as compared with no immunomodulation, respectively, over a mean follow-up of 5 months (*Figure 1C* and *Figure S9*). The sensitivity analysis after the inclusion of three trials that did not report ΔLVEF suggested no significant change in effect size or direction for mortality (*Figure S10*).

Trial sequential analysis

The cumulative Z-score to study the improvement in LVEF with immunomodulation as compared with no immunomodulation crossed the Lan–DeMets boundary for the true benefit, increasing the robustness of pooled trial results (*Figure S11*). Similar findings were seen for improvement in LVEDD (*Figure S12*). The Z-score for mortality with immunomodulation did not cross the statistical boundary of benefit or harm or futility, indicating the lack of currently available data to support conclusions regarding mortality differences (*Figure S13*).

Discussion

In this study, we observed that in patients with HFrEF, immunomodulation therapy led to a significant improvement in LVEF and reduction in LVEDD, without conclusive differences in the risk of mortality. The improvement in LVEF was seen with both anti-cytokine therapy and broad immunomodulation and was consistent across aetiologies of HF. Similarly, there was a trend towards a reduction in LVEDD with both anti-cytokine therapy and broad immunomodulation. Further, the TSA suggested a lack of sufficient extant data in these trials to demonstrate a difference in mortality with immunomodulation as compared with placebo.

Emerging evidence indicates that HF is composed of a dysregulated inflammatory milieu with altered immune cell functional networks.[1,2] A persistent inflammatory response to injury (ischaemic, infectious, pressure, or volume overload), or a failure to resolve inflammation following acute injury, can lead to chronic expression of pro-inflammatory cytokines that have been suggested to contribute to HF progression.[36] Moreover, the failing heart exhibits expanded populations and activation of both innate and adaptive immune cells that are essential contributors to progressive LV remodelling.[2,3,5,7,10] Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 are associated with increasing severity of HF[1,37,38] and contribute to progressive LV remodelling and systolic dysfunction.[39,40]

The improvement in LVEF and LVEDD seen with immunomodulation in our analyses suggests that suppression of chronic inflammation, properly targeted to the appropriate patients, may have a therapeutic benefit on LV remodelling in HFrEF. Of the 19 studies included in our meta-analysis, eight studies[23,25–27,30,31,34,35] reported changes in the levels of inflammatory cytokines TNF-α, IL-1β, or IL-6 with and without immunomodulation. Only two studies[25,30] reported significantly greater reductions in blood TNF-α levels with immunomodulatory treatment as compared with no immunomodulation. This suggests that aside from suppression of cytokine elaboration, additional mechanisms such as decreased free radical production (as seen with pentoxifylline[41]) might play a synergistic role.

Existing studies suggest that during hospitalization for acute decompensated HF, circulating IL-1β levels are associated with a higher concentration of natriuretic peptides (myocardial stretch) and troponin T (myocardial injury), along with a higher hazard of death at 1 year.[42] Further, in murine models, administration of anti-IL-1β antibody given early or late after reperfused myocardial infarction improved LV remodelling and function.[43] These findings, the benefits seen with canakinumab in reducing cardiovascular outcomes,[44] and incident HF after myocardial infarction[12] identify IL-1β as an especially promising therapeutic target for immunomodulation.

A previous meta-analysis of six studies with 221 patients (LVEF ≤ 40%) that evaluated the effect of pentoxifylline in HF suggested a 70% reduced odds of mortality as compared with placebo.[45] In our study, we grouped pentoxifylline with other anti-cytokine immunotherapy and demonstrated a comparable 51% reduced risk of mortality, although this was not statistically significant. Evidence for the use of other immunomodulatory agents is limited and has not been consistently demonstrated in humans. Anakinra (a recombinant IL-1 receptor antagonist) has been shown to reduce C-reactive protein levels and improve oxygen consumption in patients with HF.[46]

There is an increased expression of autoantibodies in human HF.[47] These may subsequently induce a persistent immune response contributing to cardiac dysfunction.[47,48] Extraction of circulating antibodies by immunoadsorption has been shown to improve cardiac index in patients with dilated cardiomyopathy.[49] Intra-venous immunoglobulin is a broad immunomodulatory agent that acts similarly to immunoadsorption and suppresses the inflammatory response via neutralization of autoantibodies and cytokines.[50] Other broad immunomodulators including prednisone, azathioprine, and methotrexate have diverse effects on both the cellular and cytokine arms of inflammation.[4]

Despite goal-directed medical therapy, HF hospitalizations and mortality remain high with nearly 40% of patients with HF succumbing to their disease within 5 years of diagnosis.[51] Current practice guidelines for HF are focused mainly on neurohormonal blockade,[52] with absolute improvements in LVEF generally less than 5% with currently approved neurohormonal blockers (i.e. angiotensin-converting enzyme inhibitors, beta-blockers, and aldosterone antagonists).[53–55] The

addition of sacubitril–valsartan on the background of optimized therapy improves LVEF ~5% after 3 months.[56] LVEF improvement with neurohormonal blockade has been suggested to reduce hospitalization and improve quality of life in patients with HF.[57,58] Our study indicates a similar improvement in LVEF with immunomodulation after a mean duration of 4.5 months as compared with placebo. While neurohormonal blockade in HF has been shown to reduce markers of inflammation,[59] it is difficult to determine whether this is an indirect result of decreased LV remodelling or a direct mechanism of action.[60] The marked LVEF improvement (~10%) seen in studies of immunomodulation in patients with non-ischaemic cardiomyopathy suggests that there may be differential therapeutic effects of immunomodulation across HF aetiologies, possibly the result of a different profile of immune system activation in non-ischaemic vs. ischaemic HF.[61–63]

Despite potential beneficial effects of immunomodulation on LV function and remodelling, such therapy has not translated into the improvement of hard cardiovascular endpoints in several trials, with some trials even suggesting harm.[11] Possible explanations for these seemingly paradoxical results may relate to treatment-related factors such as timing, duration, and type of therapy; patient-related factors such as underlying HF aetiology and co-morbidities; and the complex nature of the immune and inflammatory response itself. Indeed, while there are established detrimental effects of sustained inflammation on cardiac remodelling and function,[1,63] immunoinflammatory activation is also necessary for the initiation of reparative and defence pathways essential for restoring tissue integrity (which may be hampered by immunomodulation that is not precisely targeted).[2] Hence, a better understanding of specific immune activation pathways and their temporality after myocardial injury would be critical for eliciting beneficial effects while reducing harm after immunomodulation.

To the best of our knowledge, this is the first and largest meta-analysis inclusive of most studies aimed at evaluating the combined response of immunomodulation on LV structure, function, and outcomes in HFrEF. The observed improvements in LVEF and LVEDD suggest a beneficial reverse-remodelling effect that should be further explored in large-scale RCTs. The lack of mortality benefit could be due to competing co-morbidities, lack of precise target inhibition that increases untoward effects, or suboptimal duration of follow-up. It can also be argued that immunomodulation improves echocardiographic parameters without clinical outcome benefit. Indeed, large-scale trials of TNF neutralization failed to show benefit with regard to mortality, although changes in remodelling were not assessed.[15,26] However, as our TSA of mortality did not cross the boundary of futility, future trials should be pursued to understand whether clinical outcomes are beneficially impacted by adding immunomodulatory therapy to currently approved goal-directed medical therapy in HFrEF.

Meta-analyses have well-acknowledged limitations.[64] Heterogeneity in treatment protocols, background treatment, and patient characteristics, and short follow-up duration in the studies limit understanding of the ideal patient population that might benefit from immunomodulation. Further, besides all-cause mortality, data regarding the risk of hospitalization and cardiovascular mortality were not uniformly available for quantitative analysis. Improvement in HF outcomes has to be balanced against an increased risk of infections with immunosuppression.

Conclusions

To summarize, our meta-analysis suggests that in HFrEF patients, immunomodulation leads to a significant improvement in LV remodelling, as indexed by LVEF and LVEDD. The improvement in LVEF was seen with both anti-cytokine therapy and broad immunomodulation and across non-ischaemic and ischaemic aetiologies of HFrEF. The TSA suggested limited available data to definitively define differences in mortality risk. Future investigations are required to understand the ideal HFrEF patient population that would benefit from immunomodulation and the specific immunomodulatory therapies that should be considered.

Conflict of Interest

None of the authors had any conflicts of interest or financial disclosures to declare.

Funding

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. PRISMA checklist
Data S2. Inclusion exclusion criteria for all trials

Data S3. Search Strategy and data collection

Data S4. Statistics

Figure 1. Flow diagram for study selection

Figure 2. Risk-of-bias summary for randomized trials (RoB 2.0) assessed using the Cochrane RoB tool[28]

Figure S3. Review author's judgements about each risk-of-bias for each trial included. Green, yellow and red solid circles represent low, some concern and high risk-of-bias, respectively.

Figure S4. Effect of immunomodulation on LVEF as compared to no immunomodulation according to drug class. Black solid square diamonds and associated solid lines represent summary RR and 95% CI of each trial listed in the left column. The numerical estimates in the right columns are weighted mean difference(s) (WMD) with 95% CI of each trial listed in the left column. The hollow blue diamond is summary WMD and 95% CI for immunomodulation as compared to no immunomodulation. The hollow red diamond is the overall summary WMD with 95% CI. CI: Confidence interval

Figure S5. Funnel plot for publication bias with each blue dot representing a randomized trial and the dotted lines representing the pseudo 95% confidence intervals.

Figure S6. : Effect of immunomodulation on LVEF as compared to no immunomodulation according to heart failure aetiology. Black solid square diamonds and associated solid lines represent summary RR and 95% CI of each trial listed in the left column. The numerical estimates in the right columns are weighted mean difference(s) (WMD) with 95% CI of each trial listed in the left column. The hollow blue diamond is summary WMD and 95% CI for immunomodulation as compared to no immunomodulation. The hollow red diamond is the overall summary WMD with 95% CI. CI: Confidence interval

Table 3. Mean difference, weighted (WMD) and standardized (SMD) for primary and secondary outcomes.

Figure 7. Effect of immunomodulation on LVEDD as compared to no immunomodulation according to drug class. Black solid square diamonds and associated solid lines represent summary RR and 95% CI of each trial listed in the left column. The numerical estimates in the right columns are weighted mean difference(s) (WMD) with 95% CI of each trial listed in the left column. The hollow blue diamond is summary WMD and 95% CI for each class of immunomodulation as compared to no immunomodulation. The hollow red diamond is the overall summary WMD with 95% CI. CI: Confidence interval

Figure 8. Effect of immunomodulation on LVEDD as compared to no immunomodulation according to heart failure aetiology. Black solid square diamonds and associated solid lines represent summary RR and 95% CI of each trial listed in the left column. The numerical estimates in the right columns are weighted mean difference(s) (WMD) with 95% CI of each trial listed in the left column. The hollow blue diamond is summary WMD and 95% CI for immunomodulation

as compared to no immunomodulation. The hollow red diamond is the overall summary WMD with 95% CI.CI: Confidence interval

Figure S9. Effect of immunomodulation on mortality as compared to no immunomodulation according to heart failure aetiology. Black solid square diamonds and associated solid lines represent summary RR and 95% CI of each trial listed in the left column. The numerical estimates in the right columns are the weighted mean difference(s) (WMD) with 95% CI of each trial listed in the left column. The hollow blue diamond is a summary WMD and 95% CI for immunomodulation as compared to no immunomodulation. The hollow red diamond is the overall summary RR with 95% CI.CI: Confidence interval

Figure S10. Sensitivity analysis for effect of immunomodulation on mortality as compared to no immunomodulation according to heart failure aetiology in all trials reporting mortality data. Black solid square diamonds and associated solid lines represent summary RR and 95% CI of each trial listed in the left column. The numerical estimates in the right columns are weighted mean difference(s) (WMD) with 95% CI of each trial listed in the left column. The hollow blue diamond is summary WMD and 95% CI for immunomodulation as compared to no immunomodulation. The hollow red diamond is the overall summary RR with 95% CI.CI: Confidence interval

Figure 11. Trial sequential analysis of immunomodulation vs. no immunomodulation in patients with heart failure for improvement in left ventricular ejection fraction (LVEF). The solid black line represents the line of no difference. The green lines above and below the line of no difference represent the O'Brien-Fleming trial sequential boundary for no benefit and benefit with immunomodulation, respectively. The solid black lines are upper and lower bounds for 95% CI. The green vertical line is the required information size for conclusive meta-analyses, given two-sided α=0.05 and β=0.20. The solid blue line is Z-curve derived from a random-effects meta-analysis of individual RCTs. The inner wedge represents the O'Brien-Fleming β-spending function at 80% power. The Z-curve surpassed the trial sequential boundary and the information size, indicating a true improvement in LVEF with immunomodulation as compared to no immunomodulation.

Figure S11. Trial sequential analysis of immunomodulation vs. no immunomodulation in patients with heart failure for improvement in left ventricular ejection fraction (LVEF). The solid black line represents the line of no difference. The green lines above and below the line of no difference represent the O'Brien-Fleming trial sequential boundary for no benefit and benefit with immunomodulation, respectively. The solid black lines are upper and lower bounds for 95% C.

Figure S12 Trial sequential analysis of immunomodulation vs. no immunomodulation in patients with heart failure for improvement in left ventricle end-diastolic dimension (LVEDD). The solid black line represents the line of no difference. The

green lines above and below the line of no difference represent the O'Brien-Fleming trial sequential boundary for no benefit and benefit with immunomodulation, respectively. The solid black lines are upper and lower bounds for 95% CI. The green vertical line is the required information size for conclusive meta-analyses, given two-sided α=0.05 and β=0.20. The solid blue line is Z-curve derived from a random-effects meta-analysis of individual RCTs. The inner wedge represents the O'Brien-Fleming β-spending function at 80% power. The Z-curve surpassed the trial sequential boundary and the information size, indicating a true improvement in LVEDD with immunomodulation as compared to no immunomodulation.

Figure S13 Trial sequential analysis of immunomodulation vs. no immunomodulation in patients with heart failure for improvement in mortality. The solid black line represents the line of no difference. The solid black line represents the line of no difference. The green lines above and below the line of no difference represent the O'Brien-Fleming trial sequential boundary for no benefit and benefit with immunomodulation, respectively. The solid black lines are upper and lower bounds for 95% CI. The green vertical line is the required information size for conclusive meta-analyses, given two-sided α=0.05 and β=0.20. The solid blue line is Z-curve derived from a random-effects meta-analysis of individual RCTs. The inner wedge represents the O'Brien-Fleming β-spending function at 80% power. The Z-curve did not surpass the trial sequential boundary and the information size, indicating a lack of sufficient evidence to conclude effect on mortality with or without immunomodulation.

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