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ORIGINAL RESEARCH ARTICLE

Prednisone and azathioprine in patients with inflammatory cardiomyopathy: systematic review and meta-analysis

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Abstract

Aims Chronic non-viral myocarditis, also called inflammatory cardiomyopathy, can be treated with immune suppression on tops of optimal medical therapy (OMT) for heart failure, using a combination of prednisolone and azathioprine (IPA). However, there has been inconsistency in the effects of immunosuppression treatment. This meta-analysis is the first to evaluate all available data of the effect of this treatment on left ventricular ejection fraction (LVEF) and the combined clinical endpoint of cardiovascular mortality and/or heart transplantation-free survival.

Methods and results All trials with using IPA vs. OMT in this syndrome were searched using OVID Medline and ClinicalTrials.gov, following the PRISMA guidelines. Missing data were retrieved after contacting the corresponding authors. All data was reviewed and analysed using and standard meta-analysis methods. A random effect model was used to pool the effect sizes. A total of four trials (three randomised controlled trials and one propensity-matched retrospective registry) including 369 patients were identified. IPA on top of OMT did not improve LVEF [mean difference 9.9% (95% confidence interval −1.8, 21.7)] with significant heterogeneity. When we limited our pooled estimate to the published studies only, significant LVEF improvement by IPA was observed [14% (1.4, 26.6)]. No cardiovascular mortality benefit was observed with the intervention [risk ratio 0.34 (0.08, 1.51)].

Conclusions At the moment, there is insufficient evidence supporting functional and prognostic benefits of IPA added to OMT in virus negative inflammatory positive cardiomyopathy. Further adequate-powered well-designed prospective RCTs should be warranted to explore the potential effects of adding immunosuppressive therapy to OMT.

Keywords Myocarditis; Immunosuppression; Prednisolone; Azathioprine; Failure; Cardiomyopathy

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Introduction

Heart failure (HF) is a complex clinical syndrome, which possess a severe burden upon patients and health care systems because of decreased quality of life, frequent hospital admissions, mortality, and increasing health care resources utilisation.[1]

Although HF with reduced ejection fraction (HFrEF) can be sub categorised into ischemic and non-ischemic aetiology, this difference is not reflected in the current guidelines on HF treatment.[2]

However, for a certain portion of patients with HFrEF and persistent symptoms despite optimal guideline-based medical treatment (OMT), there is a potential therapeutic

intervention whereby immunosuppressive therapy can improve left ventricular ejection fraction (LVEF) if the HF syndrome is because of chronic non-viral inflammation.[3,4] This entity is referred as 'auto-immune' or 'inflammation positive virus negative' chronic myocarditis.

Pathophysiology of an inflammatory cardiomyopathy can be very diverse, ranging from auto-immune, toxic to infectious.[5] Only studies conducted in non-fulminant non-viral myocarditis or auto-immune myocarditis are included in this meta-analysis. This syndrome is thought to be caused by an intrinsic auto-immune disease, yet research has demonstrated that an initial viral myocarditis can also trigger pathological persistent myocardial inflammation even after the virus has been cleared.[6] Regulatory T-cells have been shown to play a key role in controlling the immune response in viral myocarditis.[7] The role of genetic susceptibility to develop a pathological response in viral myocarditis that can ultimately causes HF has been suspected in animal models.[8] The rationale of using immunosuppression in inflammatory cardiomyopathies is to halt the adverse innate immune response that causes the HF syndrome in these patients.

There has been inconsistency in the effect of immunosuppression treatment.[3–16] A recent systematic review[17] showed that recent two randomised controlled trials (RCTs) with a combination therapy of prednisolone plus azathioprine demonstrated improvement in LVEF. In addition, a large propensity-matched registry data has demonstrated for the first time prognostic improvement with the combination therapy.[16]

A systematic review and meta-analysis of trials using a combination of immune suppressive therapy with prednisone and azathioprine (IPA) to treat HF refractory to OMT caused by chronic non-fulminant virus negative or 'autoimmune' myocarditis was conducted to demonstrate the effect on LVEF and HF events.

Methods

Identification and selection of studies

Protocol and registration

The systematic review and meta-analysis adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for meta-analyses of interventional studies and its checklist.[18] Our systematic review was prospectively registered to PROSPERO (CRD42018100902, https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=100902.).[19]

Eligibility criteria

Based on our hypothesis, studies were included when they satisfied the following criteria: (i) (*P*) adults patients (>16 years old) with biopsy-proven non-infective inflammatory cardiomyopathy (defined as auto-immune or virus negative/inflammation positive) AND LVEF <50% despite OMT for >3 months. (ii) (*I*) Intervention with prednisone and azathioprine on top of the standard HF care. (iii) (*C*) Control group with standard HF care only. (iv) (O) Primary outcome as the change in LVEF. The secondary outcome was cardiovascular mortality or HTx. (v) (*S*) RCTs or observational studies including registries if they have a control group.

Information sources and search

We searched OVID MEDLINE and ClinicalTrials.gov from January 1950 till June 2018. The search terms used were: 'cardiomyopathy', 'myocarditis', 'immunosuppressive agents', 'steroids', 'prednisone', and 'azathioprine', using Mesh terms and including text search. Full details of search strategies used are presented in Appendix A. We restricted our search to human studies only (no animal experiments) with English only (language restriction). We also searched on cinicaltrials.gov for ongoing or aborted studies and contacted study authors directly when necessary.

Study selection

Identified articles via electronic database search were screened by the title and abstract level for pertinence by four independent investigators (ABP, PT, KN, and OA). Based on the previous negative findings,[9,10] we excluded those with a single agent intervention with either prednisone or azathioprine only. Remained articles were examined for inclusion based on the above a priori determined criteria with full-text reading. Review articles, editorials, letters, case reports, and case series without control were also excluded.

Data extraction

Data collection process

Data were extracted as independently by the four investigators using a dedicated data-extraction form.

Data items

We collected the information on patient characteristics, trial design, intervention details, and outcomes (*Table 1*). We also searched the 'study rationale and method papers' of selected trials to retrieve further details.[21] Our primary outcome was the changes in LVEF. The secondary outcome was cardiovascular mortality or heart transplantation free survival/HTx. When the selected outcomes were not reported in the original reports, we contacted the authors, who kindly shared the information with us.[3,4,16]

Risk of bias

Risk of bias in individual studies

We evaluated risks of bias at the study level using dedicated tools for randomised (RoB 2.0) and non-randomised

Table 1 Baseline characteristics of included studies

Study	Wojnicz	Frustaci	CZECH-ICIT[a]	Merken
Year	2001	2009	2013	2018
Study design	RCT	RCT	RCT	Registry with matched control group
LVEF inclusion threshold (%)	<40	<40	<40	Not required[b]
Duration of symptoms (months)	>6 m	>6 m	>6 m	>6 m
NYHA class	II–IV	II–IV	≥II	I–IV
Clinical FU (months)	24	10–72	12[b]	15–47
Sample size	84	85	20	180
Mean age (years)	40	43	NA	49
LVEF baseline (%)				
Control	23.8 ± 8.6	27.7 ± 6.4	22.2 ± 3.7	33 ± 14
Intervention	24.9 ± 7.3	26.5 ± 6.6	22.3 ± 4.7	31 ± 12
Atrial fibrillation (%)	30	NA	NA	15
Left bundle branch block (%)	NA	16	NA	20
IPA: prednisone				
Starting dose	1 mg/kg/day for 12 days	·Arm A: Wojnicz protocol	·Arm A: Wojnicz protocol	·1 mg/kg/day for 4 weeks
Maintenance dose	·Every 5 days, tapered by 5 mg to 0,2 mg/day	·Arm B: Frustaci protocol	·Arm B: Frustaci protocol	·0,33 mg/kg/day afterwards
IPA: azathioprine (mg/kg/day)	1 mg/kg/day	2 mg/kg/day	1–2 mg/kg/day	2 mg/kg/day
IPA: total duration (months)	3 m	6 m[c]	3–6 m	6 m[d]
Primary outcome	Mortality, HTx, or HF readmission	Change in LVEF	Change in LVEF	Mortality or HTx
Result primary outcome	Negative	Positive	Negative	Positive
Secondary outcome	Change in LVEF	Mortality or HTx	Clinical events	Change in LVEF
Result secondary outcome	Positive	Negative	Negative	Positive

[a]This is a preliminary data from CZECH-ICIT (Czech Inflammatory Cardiomyopathy Immunosuppression Trial). Data on LVEF on baseline and 6 months FU and clinical events at 24 months were obtained via direct communication with the authors. Details are provided in table 2 (cf. Appendix).
[b]Merken *et al*. used European Society of Cardiology (ESC) criteria for myocarditis[20] which does not include any criteria for LVEF.
[c]Three patients received continuation of azathioprine after 6 months because of persistent/recurrent myocardial inflammation at follow-up biopsy at 6 months—a prespecified follow-up procedure.
[d]Depending on the immunologic profile, cyclosporine was added in 11 patients for at least 6 months.

intervention (ROBINS-I in non-randomised studies).[20,22,23] We extracted information on (i) randomisation, (ii) deviations from planned interventions, (iii) missing data, (iv) assessment methods for the endpoint, and (v) choice of reported results. Also, we performed a quality assessment for the trial registration protocol and study design.[21] The information was used to divide the studies for subgroup analysis in data synthesis when meaningful.

Risk of bias across studies

Publication bias was assessed visually using funnel plots.

Qualitative analysis

Summary measures

Mean differences (MDs) were used for assessing the change in LVEF and risk ratios (RRs) for binary outcomes, both with their 95% confidence intervals (CIs).

Synthesis of results

The pooled MD and RR were calculated using a random effects model; DerSimonian–Laird method[24] because we assumed that the true effect sizes for these studies would be distributed around a mean, instead of the fixed true value. Heterogeneity was assessed using I^2 and Cochran Q tests. 'I^2 values of 25%, 50%, and 75% were considered low, moderate, and high levels of heterogeneity, respectively'[27]. A Cochran Q P-value of <0.05 was considered significant, indicating heterogeneity.

Additional analysis

Prespecified subgroup analysis was conducted, dividing studies based on the studies design (RCT or not), publication status (published or not). A meta-regression was not performed because less than 10 studies were identified.

Statistical analysis, including testing for heterogeneity and publication bias, was performed using STATA version 15.1

(StataCorp LLC, TX, USA) using the 'metan', 'funnel', and 'confunnel' packages. A *P*-value of <0.05 was considered statistically significant.

Results

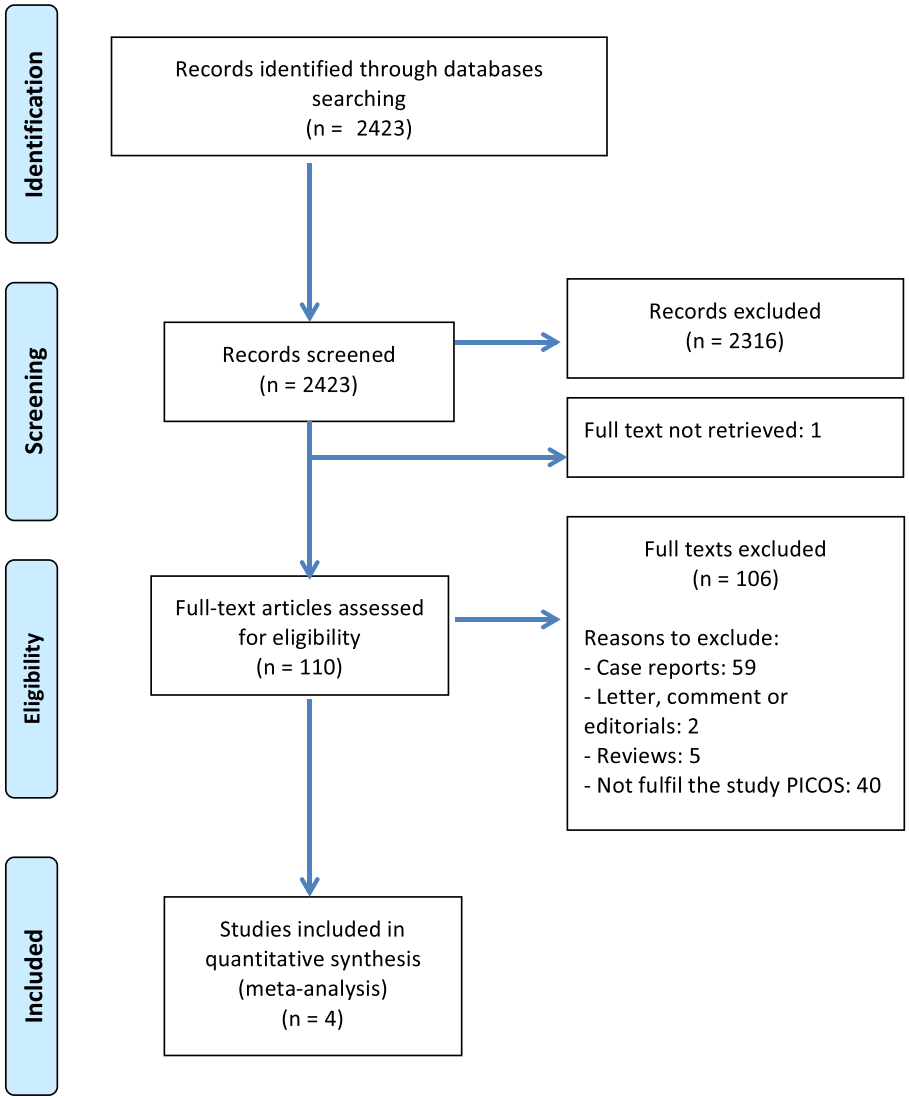
Identified and eligible studies (study selection)

The process of article selection based on PRISMA guidelines (PRISMA flow chart) is presented in *Figure 1*. A total of 121 articles were identified via the database search (MEDLINE and Clinical Trials.gov), and additional 10 studies were from the previous report[18] (which initially identified 8087 articles via PubMed (*n* = 4823), Embase (*n* = 2830), LILAC (*n* = 294), and Cochrane library (*n* = 140) by the search until January 2016). There were no duplicates. The titles and abstracts of 131 articles were screened for eligibility. Among them, 110 were included and assessed by full text, and 106 were excluded because of the following reasons: case report (*n* = 59), Letter/editorial (*n* = 2), reviews (*n* = 5), and not fulfilling our PICO (*n* = 40). A total of four articles (369 patients) were eligible for the qualitative synthesis and meta-analysis.

Characteristics of included studies

Baseline demographics of the four included studies are summarised in *Table 1*. Two of them were published RCTs,[3,4]

Figure 1 PRISMA flow chart.



one was a published registry with a corresponding control group,[16] and one with aborted RCT without publication.[21] Their populations were small to mid in size between 20[21] and 180[16] with a total of 369 patients. The participants were all adults with biopsy-proven inflammatory cardiomyopathy, and received either of additional IPA to standard HF care, or standard HF care only. All of them had HF symptoms for more than 6 months before the enrolment of the study but one study,[16] which included 53 patients (29%) with NYHA class I at the time of enrolment. Follow-up periods were different for LVEF assessment and clinical endpoints. LVEF was measured after 6 months in the RCTs[3,4,21] and 12 months in the registry.[16] Because there is no evidence of any further increase in left LVEF beyond 6 months after treatment in the individual trials,[3,4] the FU value was chosen at 6 months. Cardiovascular endpoints were either mortality or HTx. Both the Frustaci and Wojnicz group assessed the endpoints at 2 years after initiation of therapy; in the Merken registry, the time window of clinical follow-up was less well defined (median 31 months, range 15–47). All endpoints were assessed together in the present analysis, regardless of timing.

Weighted mean EF at baseline was 23% (range 22–32%). For the secondary outcome, three studies had statistically non-significant results, but one study showed statistically significant favourable prognostic effect.[16] The total number of events was small [n = 33 (9.6%)], which yielded wide CIs.

Risk of bias within studies

All four selected studies underwent critical appraisal using tools according to study design by all four investigators individually. Upon disagreement, consensus was reached after careful deliberation.

The summary of risk of bias about 3 RCTs is presented in Appendix B with detailed comments. First, for randomisation, all three RCTs used true random processes to generate study groups. However, only two of them reported information on allocation concealment,[3,4] resulting two studies with low risk of bias and one study with high risk of bias. In the next step, the deviations from intended interventions were reviewed, and all were deemed low risk of bias. While all clinical follow-up data were available from Wojnicz *et al.*, some of the echocardiographic data were missing. Regarding measurement of the outcome, one had high risk of bias because of open-label study design[3] whereas the rest two had low risk of bias. Finally, all selected RCTs had low risk in the selection of the reported result category. To conclude, the overall risk of bias was one with low risk,[4] another with some concerns,[3] and one with high risk of bias given limited access to the information because of unpublished nature.[21]

The risk of bias of the sole non-randomised intervention study[16] had four 'low' and three 'moderate' risks out of seven components of bias assessment by ROBINS-I in non-randomised studies template (Appendix C). The overall bias assessment of this study was judged as 'moderate' given choice of immunosuppression therapy in the treatment group is not stated, but baseline characteristics were matched. There was likely significant bias for echocardiographic measurements because both patients and operators were not blinded.

Risk of bias across studies

Both ordinary funnel and confunnel plots were used to assess the publication bias among studies (*Figure 2*). Significant asymmetric distributions of studies were observed in the change in LVEF in both funnel and confunnel plots. The distribution of the CV endpoints was showed small but obvious asymmetry. Studies with a larger standard error with borderline to negative effects were missing. Because of the low number of studies, the funnel plots are only indicative of potential bias.

Meta-analysis

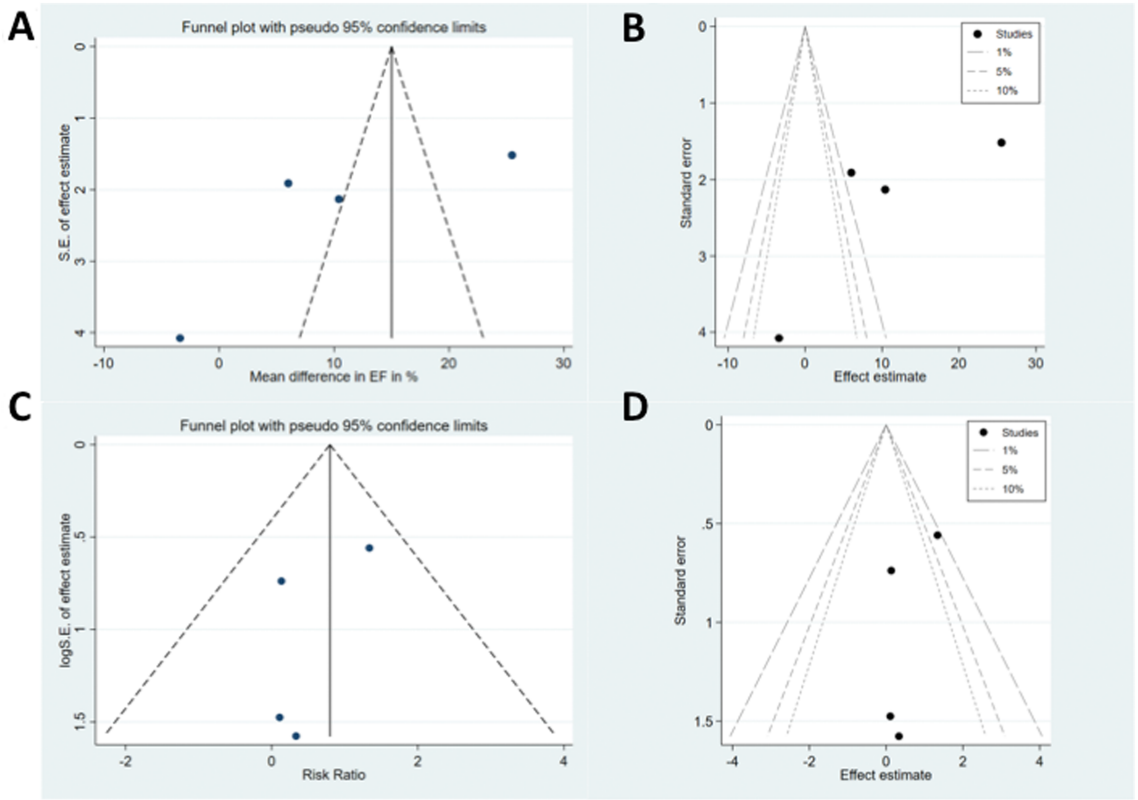
The change in left ventricular ejection fraction

Among four identified studies, all showed a significant improvement in LVEF (with a maximum increase of 25.5%[4] except one,[21] which yielded a statistically neutral result with deterioration by the point estimate of −3.4% [95% CI −11, 4.6]. Pooled mean difference in LVEF was 9.9% [−1.8, 21.7] (*Figure 3A*). Because there was significant heterogeneity (I^2 96.8%, $P < 0.001$), subgroup analyses were performed by study design to elucidate the sources of the heterogeneity. First, the studies were divided by randomisation status (*Figure 3B*), where I^2 values remained similar (96.9%). Then, they were subgrouped by publication status (*Figure 3C*), which did not reduce I^2 values, but the pooled estimates of the change in LVEF became significantly positive with 14% [1.4, 26.6].

The cardiovascular mortality

Our secondary outcome, the combined endpoint of cardiovascular mortality or HTx, was assessed in a similar manner (*Figure 4A*). Only one study demonstrated statistically significant prognostic improvement,[12] with the rest showing neutral results.[3,4,21] The pooled RR was 0.34 [0.08, 1.51]. Because of a moderate heterogeneity (I^2 = 60%), we

Figure 2 Funnel plots for publication bias. Publication bias was assessed using funnel plots. The weighted mean difference in EF (A) and (B). Cardiovascular mortality (C) and (D).



performed subgroup analyses to assess the possible contributors. When categorised by randomisation or not, an I^2 value significantly dropped close to low level (32%) with no difference in the pooled RR of the RCTs (*Figure 4B*). Subgrouping by publication status increased the heterogeneity, just missing the mark to be marked as 'high' (72.9%; *Figure 4C*).

Discussion

Statement of principal findings

Our results in the pooled analysis demonstrated that additional immunosuppression therapy with prednisone and azathioprine (IPA) to current standard HF care did not improve LVEF or clinical outcomes. However, when we limited the studies published in the literature, there was a significant improvement in LVEF. Although not statistically significant, a trend towards lower cardiovascular mortality or HTx was also observed. The heterogeneity of this result was mitigated when we analysed the clinical outcomes data from the RCTs separately. Given large heterogeneity among the identified studies, our results have to be interpreted carefully and merely as hypothesis generating.

Strengths and weaknesses of the study

The strengths of this study were four-fold: (i) analysis of the combination therapy of prednisone and azathioprine in addition to standard HF therapy in treating virus negative inflammatory positive cardiomyopathy, instead of accumulating the evidence from variety of single or dual agents and myocarditis in general; (ii) the use of surrogate (i.e. LVEF) combined with clinical endpoints (aka cardiovascular mortality and HTx-free survival); (iii) sound qualitative analysis with random effect model and subgroup analysis, providing further insights into the possible sources of heterogeneity; (iv) appropriately evaluated risk of bias using recommended tools.

Strengths and weaknesses in relation to other studies

There are two previous meta-analyses on this topic.[17,25] Only one showed improvement in EF using a fixed-effect model to

Figure 3 Forest plots on the changes in LVEF. Forest plots on the changes of EF in three different settings: (A) all four studies, (B) subgrouped by study design (RCT or not), and (C) subgrouped by publication status (published or not).

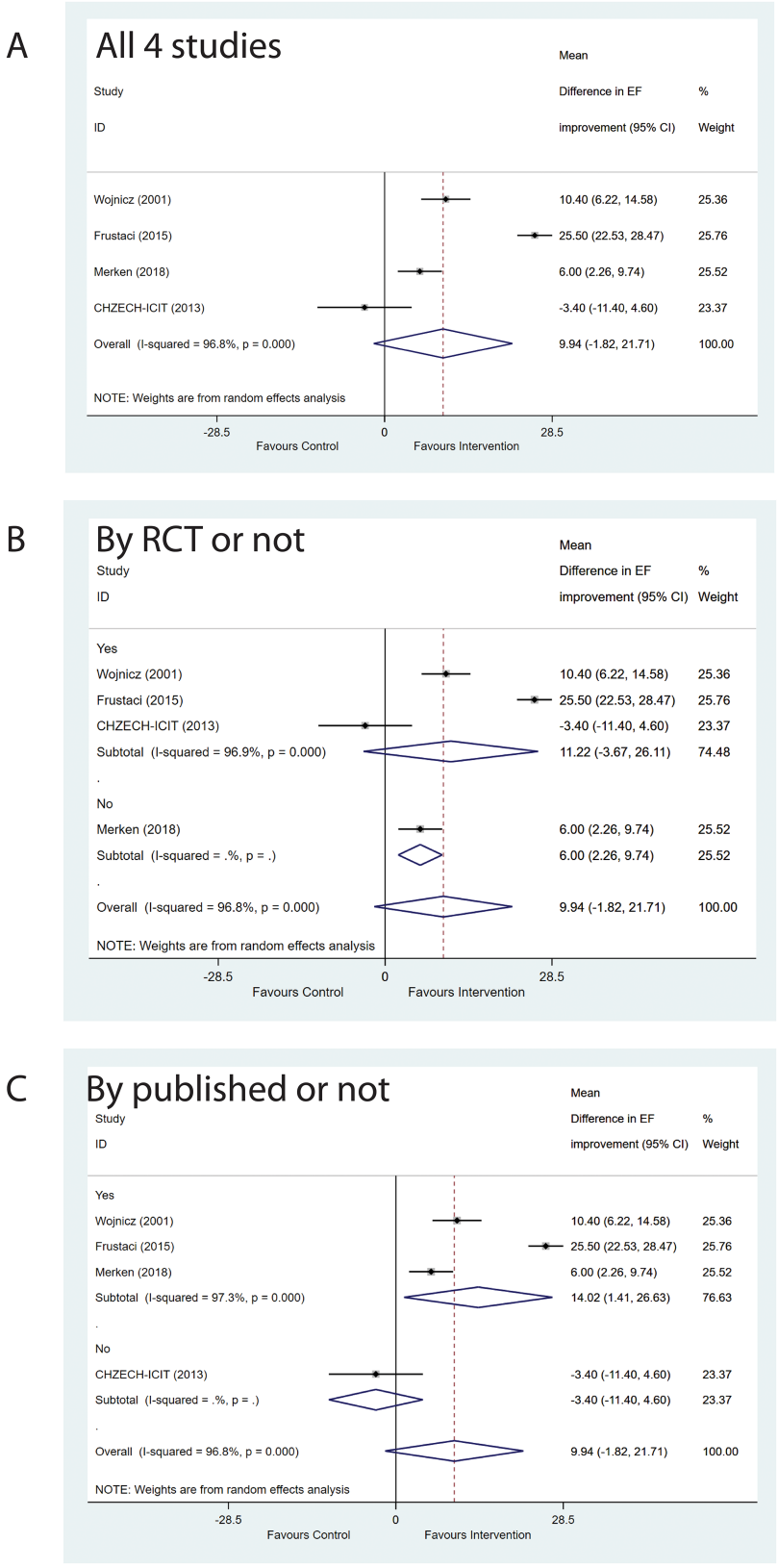
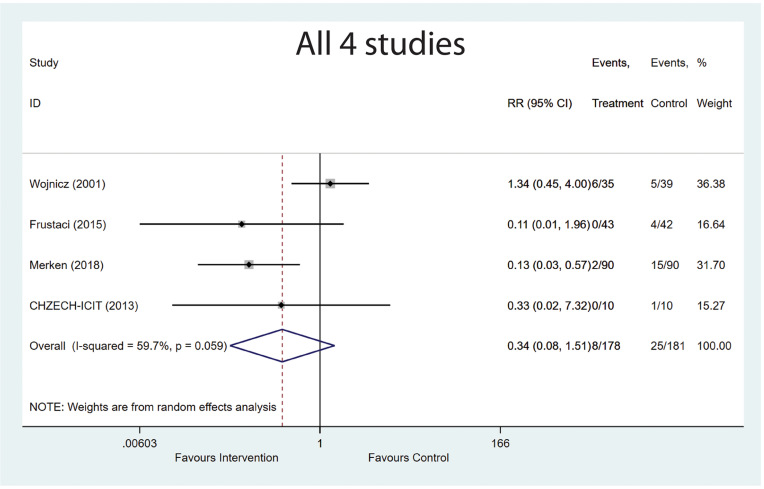
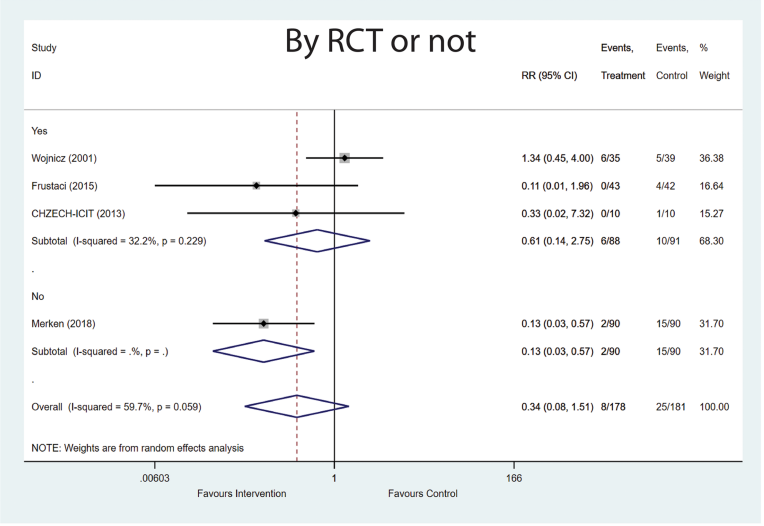


Figure 4 Forest plots on cardiovascular mortality

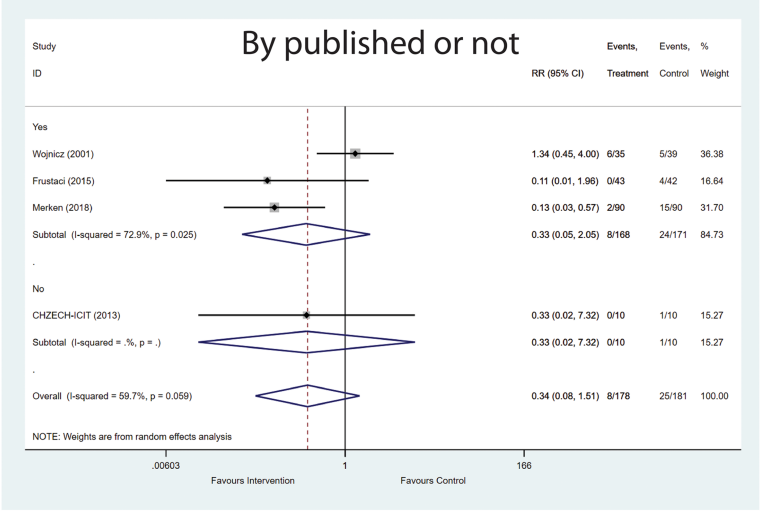
A



B



C



pool the estimate.[25] Our findings support the positive trend demonstrated in the second one,[17] where, similar to our analysis, a random effect model was used. The rationale behind using the random effect model is that the true impact of IPA would be distributed around a mean, which in this analysis is because of the heterogeneity of trial design and outcome measures. The previous two meta-analyses included only RCT or quasi-RCT design studies, which provide better internal validity by the design per se at the cost of a smaller sample size. We included one well-designed registry data matched with propensity-matched controls,[16] which included the largest patient population so far, and also data from an unpublished RCT,[21] which added the largest sample size with higher precision. This meta-analysis was not the first to analyse clinical endpoints. However, by using a larger sample size, this study allowed investigators to appreciate the effects of IPA on a larger scale.

Limitations

Our results should be interpreted in the following limitations.

Risk of bias within and across studies

Possible within-study risk of bias was identified in the unpublished RCT,[21] given the limited information provided by the authors. Across studies, meta-bias was graphically assessed. Significant asymmetrical distributions of studies observed in the funnel plots of the change in LVEF suggests the possibility of publication bias. However, the sporadic distribution made it difficult or even futile to indicate where or which direction the publication bias might sit. This was corroborated by the difference in the centre lines of funnel and confunnel plots. Because of the small number of studies identified, results of this meta-analysis should be interpreted with caution.

Heterogeneity

We also found significant heterogeneity among the four identified studies. In LVEF outcome, 96.8% of the variability in the point estimate is because of true heterogeneity rather than sampling error. This is possibly related to the different IPA regimes and inclusion criteria (see *Table 1*). Meta-regression analysis was not performed because of the small number of studies included. Furthermore, NNT was not explored because of neutral findings.

Future directions

According to this meta-analysis, additional immunosuppression therapy with IPA to current standard HF care in patients with virus negative/auto-immune inflammatory cardiomyopathy can be considered. There is no sign of harm, and a potential positive effect on both LVEF and clinical endpoints is noted.[26] Current practice will hardly be changed by this result, because the current guidelines on HF treatment are based on quantification of LVEF, regardless of aetiology.[2] However, a recent landmark trial[27] is challenging the current role of ICD implantations in non-ischemic HFrEF patients.[28,29] Moreover, diagnosing a patient reliably as having a LVEF of less than 35% is not as straightforward as previously assumed.[30] More aetiology-based therapeutic options are therefore a promising lead into the future of HF research.[31] Given the modifiable nature of the disease with favourable findings in this meta-analysis, further well-designed and adequately powered prospective RCTs in inflammatory cardiomyopathies can elucidate the true impact of immunosuppression. To prevent further heterogeneity in the results of such trials, the most promising medication scheme[4,16] should be adopted in order to assess the effect on functional outcomes combined with an adequate and standardised follow-up time to record an effect on clinical outcomes. Unfortunately, one such trial was aborted because of lack of funding (INFLAMMACOR, see Appendix D).

Conclusions

There is currently insufficient evidence supporting functional and prognostic benefits of combination therapy with prednisone and azathioprine to standard HF care in patients with inflammatory cardiomyopathy. However, given large heterogeneity and small to midsized studies, future well-designed prospective RCTs may explore the positive signal on LVEF and clinical outcomes associated with IPA to standard HF treatment that is demonstrated in this meta-analysis.

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Conflict of interest

None declared.

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Appendix A: Scientific search details

- Medline OVID
- Myocarditis

(exp Myocarditis/) OR (cardiomyopathies/ or exp cardiomyopathy, dilated/)

- Immunosuppresive

(exp Immunosuppressive Agents) OR (exp Steroids/) OR (exp Prednisolone/) OR (exp Azathioprine/)

Keyword

Appendix B: Critical appraisal of the RCTs included in the meta-analysis using the RoB 2.0 tool

B.1 Bias assessment of the Wojnicz paper

Domain	Signalling questions	Response options	Description/ support for judgement
Bias arising from the randomisation process	1.1 Was the allocation sequence random?	Y / PY / PN / N / NI	Y
	1.2 Was the allocation sequence concealed until participants were recruited and assigned to interventions?	Y / PY / PN / N / NI	NI
	1.3 Were there baseline imbalances that suggest a problem with the randomisation process?	Y / PY / PN / N / NI	N
	Risk of bias judgement	Low / High / Some concerns	Some concerns
	Optional: What is the predicted direction of bias arising from the randomisation process?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable	Towards null
Bias because of deviations from intended interventions	2.1. Were participants aware of their assigned intervention during the trial?	Y / PY / PN / N / NI	NI
	2.2. Were carers and trial personnel aware of participants' assigned intervention during the trial?	Y / PY / PN / N / NI	NI
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention beyond what would be expected in usual practice?	NA / Y / PY / PN / N / NI	N
	2.4. If Y/PY to 2.3: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?	NA / Y / PY / PN / N / NI	
	2.5 Were any participants analysed in a group different from the one to which they were assigned?	Y / PY / PN / N / NI	N
	2.6 If Y/PY/NI to 2.5: Was there potential for a substantial impact (on the estimated effect of intervention) of analysing participants in the wrong group?	NA / Y / PY / PN / N / NI	
	Risk of bias judgement	Low / High / Some concerns	Some concerns
	Optional: What is the predicted direction of bias because of deviations from intended interventions?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable	no
Bias because of missing outcome data	3.1 Were outcome data available for all, or nearly all, participants randomised?	Y / PY / PN / N / NI	PN
	3.2 If N/PN/NI to 3.1: Are the proportions of missing outcome data and reasons for missing outcome data similar across intervention groups?	NA / Y / PY / PN / N / NI	PY
	3.3 If N/PN/NI to 3.1: Is there evidence that results were robust to the presence of missing outcome data?	NA / Y / PY / PN / N / NI	PN
	Risk of bias judgement	Low / High / Some concerns	Some concerns
	Optional: What is the predicted direction of bias because of missing outcome data?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable	
		Y / PY / PN / N / NI	PY

(*Continues*)

Domain	Signalling questions	Response options	Description/ support for judgement
Bias in measurement of the outcome	4.1 Were outcome assessors aware of the intervention received by study participants?		
	4.2 If Y/PY/NI to 4.1: Was the assessment of the outcome likely to be influenced by knowledge of intervention received?	NA / Y / PY / PN / N / NI	PY
	Risk of bias judgement	Low / High / Some concerns	Some concerns
	Optional: What is the predicted direction of bias because of measurement of the outcome?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable	Favours experimental
Bias in selection of the reported result	Are the reported outcome data likely to have been selected, on the basis of the results, from…		N
	5.1. … multiple outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	Y / PY / PN / N / NI	
	5.2 … multiple analyses of the data?	Y / PY / PN / N / NI	N
	Risk of bias judgement	Low / High / Some concerns	low
	Optional: What is the predicted direction of bias because of selection of the reported result?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable	
Overall bias	**Risk of bias judgement**	Low / High / Some concerns	Some concerns
	Optional: What is the overall predicted direction of bias for this outcome?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable	Favours experimental

B.2 Bias assessment of the Frustaci paper using the RoB 2.0 tool

Domain	Signalling questions	Response options	Description/ support for judgement
Bias arising from the randomisation process	1.1 Was the allocation sequence random?	Y / PY / PN / N / NI	Y
	1.2 Was the allocation sequence concealed until participants were recruited and assigned to interventions?	Y / PY / PN / N / NI	Y
	1.3 Were there baseline imbalances that suggest a problem with the randomisation process?	Y / PY / PN / N / NI	N
	Risk of bias judgement	Low / High / Some concerns	low
	Optional: What is the predicted direction of bias arising from the randomisation process?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable	Towards null
Bias because of deviations from intended interventions	2.1. Were participants aware of their assigned intervention during the trial?	Y / PY / PN / N / NI	N
	2.2. Were carers and trial personnel aware of participants' assigned intervention during the trial?	Y / PY / PN / N / NI	N
	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention beyond what would be expected in usual practice?	NA / Y / PY / PN / N / NI	

(*Continues*)

Domain	Signalling questions	Response options	Description/ support for judgement
	2.4. If Y/PY to 2.3: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?	NA / Y / PY / PN / N / NI	
	2.5 Were any participants analysed in a group different from the one to which they were assigned?	Y / PY / PN / N / NI	N
	2.6 If Y/PY/NI to 2.5: Was there potential for a substantial impact (on the estimated effect of intervention) of analysing participants in the wrong group?	NA / Y / PY / PN / N / NI	
	Risk of bias judgement	Low / High / Some concerns	low
	Optional: What is the predicted direction of bias because of deviations from intended interventions?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable	
Bias because of missing outcome data	3.1 Were outcome data available for all, or nearly all, participants randomised?	Y / PY / PN / N / NI	Y
	3.2 If N/PN/NI to 3.1: Are the proportions of missing outcome data and reasons for missing outcome data similar across intervention groups?	NA / Y / PY / PN / N / NI	
	3.3 If N/PN/NI to 3.1: Is there evidence that results were robust to the presence of missing outcome data?	NA / Y / PY / PN / N / NI	
	Risk of bias judgement	Low / High / Some concerns	low
	Optional: What is the predicted direction of bias because of missing outcome data?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable	
Bias in measurement of the outcome	4.1 Were outcome assessors aware of the intervention received by study participants?	Y / PY / PN / N / NI	N
	4.2 If Y/PY/NI to 4.1: Was the assessment of the outcome likely to be influenced by knowledge of intervention received?	NA / Y / PY / PN / N / NI	N
	Risk of bias judgement	Low / High / Some concerns	low
	Optional: What is the predicted direction of bias because of measurement of the outcome?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable	
Bias in selection of the reported result	Are the reported outcome data likely to have been selected, on the basis of the results, from…		N
	5.1. … multiple outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	Y / PY / PN / N / NI	
	5.2 … multiple analyses of the data?	Y / PY / PN / N / NI	N
	Risk of bias judgement	Low / High / Some concerns	low
	Optional: What is the predicted direction of bias because of selection of the reported result?	Favours experimental / Favours comparator /	Towards null

(*Continues*)

Domain	Signalling questions	Response options	Description/ support for judgement
		Towards null / Away from null / Unpredictable	
Overall bias	**Risk of bias judgement**	Low / High / Some concerns	low
	Optional: What is the overall predicted direction of bias for this outcome?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable	

Appendix C: Bias assessment of the registry included in the meta-analysis using the ROBINS-I tool

C.1 Bias assessment of the Merken paper using the ROBINS-I tool

	Signalling questions	Description	Response options
Bias because of confounding			
	1.1 Is there potential for confounding of the effect of intervention in this study? If N/PN to 1.1: the study can be considered to be at low risk of bias because of confounding and no further signalling questions need be considered If Y/PY to 1.1: determine whether there is a need to assess time-varying confounding:	N (propensity matching score) Yes	Y / PY / PN / N
	1.2. Was the analysis based on splitting participants' follow-up time according to intervention received? If N/PN, answer questions relating to baseline confounding (1.4 to 1.6) If Y/PY, go to question 1.3.	Yes (cohort)	NA / Y / PY / PN / N / NI
	1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome? If N/PN, answer questions relating to baseline confounding (1.4 to 1.6) If Y/PY, answer questions relating to both baseline and time-varying confounding (1.7 and 1.8)	No	NA / Y / PY / PN / N / NI
	Questions relating to baseline confounding only		
	1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	Yes	NA / Y / PY / PN / N / NI
	1.5. If Y/PY to 1.4: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	Propensity matching, thereby excluding some patients	NA / Y / PY / PN / N / NI
	1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	NI	NA / Y / PY / PN / N / NI

(*Continues*)

Signalling questions	Description	Response options
Bias because of confounding		
Questions relating to baseline and time-varying confounding		
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	PN	NA / Y / PY / PN / N / NI
1.8. If Y/PY to 1.7: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?		NA / Y / PY / PN / N / NI
Risk of bias judgement	low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias because of confounding?		Favours experimental / Favours comparator / Unpredictable
Bias in selection of participants into the study		
2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? If N/PN to 2.1: go to 2.4	NI	Y / PY / PN / N / NI
2.2. If Y/PY to 2.1: Were the post-intervention variables that influenced selection likely to be associated with intervention?	N	NA / Y / PY / PN / N / NI
2.3 If Y/PY to 2.2: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?		NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Y	Y / PY / PN / N / NI
2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?		NA / Y / PY / PN / N / NI
Risk of bias judgement	low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias because of selection of participants into the study?	unpredictable	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable
Bias in classification of interventions		
3.1 Were intervention groups clearly defined?	N	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Y	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	PY	Y / PY / PN / N / NI
Risk of bias judgement	moderate	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias because of classification of interventions?	unpredictable	Favours experimental / Favours comparator / Towards null /Away

(*Continues*)

Signalling questions	Description	Response options
Bias because of confounding		
		from null / Unpredictable
Bias because of **deviations from intended interventions**		
If your aim for this study is to assess the effect of assignment to intervention, answer questions 4.1 and 4.2		
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	N	Y / PY / PN / N / NI
4.2. **If Y/PY to 4.1**: Were these deviations from intended intervention unbalanced between groups *and* likely to have affected the outcome?		NA / Y / PY / PN / N / NI
If your aim for this study is to assess the effect of starting and adhering to intervention, answer questions 4.3 to 4.6		
4.3. Were important co-interventions balanced across intervention groups?	N	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Y	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	NI	Y / PY / PN / N / NI
4.6. If N/PN to 4.3, 4.4 or 4.5: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?	N	NA / Y / PY / PN / N / NI
Risk of bias judgement	Moderate (no info on drug adherence)	
Optional: What is the predicted direction of bias because of deviations from the intended interventions?	Favours comparator (=OMT without immunosuppression)	
Bias because of missing data		
5.1 Were outcome data available for all, or nearly all, participants?	Y	Y / PY / PN / N / NI
5.2 Were participants excluded because of missing data on intervention status?	N	Y / PY / PN / N / NI
5.3 Were participants excluded because of missing data on other variables needed for the analysis?	PN	Y / PY / PN / N / NI
5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?		NA / Y / PY / PN / N / NI
5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?		NA / Y / PY / PN / N / NI
Risk of bias judgement	low	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias because of missing data?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable
Bias in measurement of outcomes		
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	PY (for echocardiographic measurements) – not for mortality or HTx!	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	PY (for echocardiographic measurements) – not for mortality or HTx!	Y / PY / PN / N / NI

(*Continues*)

	Signalling questions	Description	Response options
Bias because of confounding			
	6.3 Were the methods of outcome assessment comparable across intervention groups?	Y	Y / PY / PN / N / NI
	6.4 Were any systematic errors in measurement of the outcome related to intervention received?	N	Y / PY / PN / N / NI
	Risk of bias judgement	Moderate (for EF) – low for mortality or HTx!	Low / Moderate / Serious / Critical / NI
	Optional: What is the predicted direction of bias because of measurement of outcomes?	Favours experitmental	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable
Bias in selection of the reported result			
	Is the reported effect estimate likely to be selected, on the basis of the results, from…	N	
	7.1. … multiple outcome *measurements* within the outcome domain?		Y / PY / PN / N / NI
	7.2 … multiple *analyses* of the intervention-outcome relationship?	N	Y / PY / PN / N / NI
	7.3 … different *subgroups*?	N	Y / PY / PN / N / NI
	Risk of bias judgement	low	Low / Moderate / Serious / Critical / NI
	Optional: What is the predicted direction of bias because of selection of the reported result?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable
Overall bias			
	Risk of bias judgement	Moderate; choice of immunnosuprression therapy in treatment group is not stated, but baseline characteristics are matched. There is likely bias for echocardiographic measurements because patients nor operators were not blinded.	Low / Moderate / Serious / Critical / NI
	Optional: What is the overall predicted direction of bias for this outcome?		Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable

Appendix D: The INFLAMMACOR project

The INFLAMMACOR trial a project managed by the cardiomyopathy research team at the Maastricht University (the Netherlands) and is planned with a prospective randomised open-label blinded end-point (PROBE) design. Target group are patients with a biopsy proven inflammatory non-ischemic non-valvular cardiomyopathy not responding to conventional optical medical therapy after 3 months. The syndrome is defined as follows:

- LVEF $\leq$ 40% (also non-dilated) despite optimal HF therapy for 3 months
- Non-significant viral load ($<$500 copies per microgram DNA, PVB19, HHV4, and HHV6)
- EMB: CD45 $\geq$ 14 with the presence of up to 4 CD68 or CD3 $\geq$7 cells/mm^2

The intervention is the addition of a combined immunosuppressive treatment for 6 months on top of conventional heart failure drugs. The dose regime consists of prednisone (1 mg/kg/day first 4 weeks, 0.33 mg/kg/day afterwards) and azathioprine (2 mg/kg/day).

The primary endpoint is the change in LVEF after 6 and 12 months as assessed with echocardiography. Secondary endpoint is a combined endpoint of overall mortality, (cardiovascular) hospitalisation, heart transplantation, ICD shocks/resuscitation, NYHA class, LV chamber dimensions (LVEDD, LVESD), (opportunistic) infections, 6-min walking distance, Minnesota Living With Heart Failure score.

A total of 80 patients (40 in both groups) are needed according to the power calculation of the study to measure a difference in the primary outcome. Seven hospitals across the Netherlands and Belgium have committed to participate.

D.1 List of participating centres for the INLAMMACOR project

Hospital	Country	Principal investigator(s)
Maastricht University Medical Centre	NL	Stephane Heymans
Groningen University Medical Centre	NL	Rudolf de Boer
OLV Aalst	B	Ward Heggermont
Jessa Hospital Hasselt	B	Philippe Timmermans, Paul Dendale
University Hospital Antwerp (UZA)	B	Constantijn Franssen, Emeline van Craenenbroeck
University Hospital Louvain (UCL)	B	Anne Catherine Pouleur
University Hospital Liège (CHL, 'Citadelle')	B	Pierre Troisfontaines

B, Belgium; NL, the Netherlands.

Funding is originally requested at the BeNeFit organisation (https://kce.fgov.be/nl/benefit-2018) but unfortunately not granted. Other sources of funding are currently being searched.

Upon contacting the research team at St. Anne's University Hospital Brno, Czech Republic regarding their experience and data of the CZECH-ICIT study, renewed interest in participation in a clinical trial was shown. The researchers contacted the PI at the Maastricht University for further collaboration and participation in the INFLAMMACOR project.