Original Article

Qiliqiangxin Alleviates Imbalance of Inflammatory Cytokines in Patients with Dilated Cardiomyopathy: A Randomized Controlled Trial*

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[Abstract] Objective: Qiliqiangxin (QLQX) capsule, a traditional Chinese medicine used for treating heart failure (HF), can modulate inflammatory cytokines in rats with myocardial infarction. However, its immune-regulating effect on dilated cardiomyopathy (DCM) remains unknown. The aim of this study was to investigate whether QLQX has a unique regulatory role in the imbalance of pro- and anti-inflammatory cytokines in patients with DCM. **Methods:** The QLQX-DCM is a randomized, double-blind trial conducted at 24 tertiary hospitals in China. A total of 345 patients with newly diagnosed virus-induced DCM were randomly assigned to receive QLQX capsules or placebo while receiving optimal medical therapy for HF. The primary endpoints were changes in plasma inflammatory cytokines and improvements in left ventricular ejection fraction (LVEF) and left ventricular end-diastolic diameter (LVEDd) over the 12-month treatment. **Results:** At the 12-month follow-up, the levels of IFN-γ, IL-17, TNF-α, and IL-4 decreased significantly, while the level of IL-10 increased in both groups compared with baselines (all $P<0.0001$). Furthermore, these changes, coupled with improvements in LVEF, NT-proBNP and New York Heart Association (NYHA) functional classification, excluding the LVEDd in the QLQX group, were greater than those in the placebo group (all $P<0.001$). Additionally, compared with placebo, QLQX treatment also reduced all-cause mortality and rehospitalization rates by 2.17% and 2.28%, respectively, but the difference was not statistically significant. **Conclusion:** QLQX has the potential to alleviate the imbalance of inflammatory cytokines in patients with DCM, potentially leading to further improvements in cardiac function when combined with anti-HF standard medications.

Keywords: Qiliqiangxin capsule; dilated cardiomyopathy; inflammatory cytokines; cardiac function; randomized controlled trial

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Dilated cardiomyopathy (DCM) is a prevalent cause of heart failure (HF) characterized by dilation of the left or both ventricles and systolic dysfunction. Due to the complexity of its etiology and pathogenesis, there is currently no specific treatment available[1]. Although standard treatment with angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs), beta-blockers (β-blockers), and mineralocorticoid receptor antagonists (MRAs), among others, is known to reduce the risk of death and hospitalization in patients with HF, the morbidity and mortality of HF, including DCM, remain high[2, 3].

In recent decades, immune disorders triggered by viral infection have been shown to be closely associated with the development of DCM[4]. $CD4^+$ Th cells play important roles in the immune response by secreting their related cytokines. Previous studies have shown that Th1-mediated cellular immunity contributes significantly

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to early cardiac injury, whereas the Th2-mediated humoral immune response is predominant in the later stages of damage, leading to the occurrence of DCM[5–7]. Subsequently, Th17 cells and IL-17 were also found to be highly expressed in patients with viral myocarditis (VMC) and DCM. They participate in the progression of VMC to DCM by promoting myocardial inflammation, anti-heart autoantibody (AHA) production, virus replication, and cardiac fibrosis[8–10]. The protective effects of anti-CD4 or anti-IL-17 monoclonal antibodies (mAbs) in DCM animals suggested that correcting Th cell-secreted cytokine imbalances is critical and will likely improve outcomes in patients with DCM[10–12]. However, the efficiency and safety of previous clinical trials targeting proinflammatory cytokines in patients with HF or DCM were unsatisfactory[13].

Qiliqiangxin (QLQX) capsule is a traditional Chinese medicine (TCM) derived from 11 different herbal extracts, as detailed in table S1. Notably, *Astragali radix* and *Aconiti lateralis radix preparata* are the primary pharmacologically active components[14]. A series of clinical studies have demonstrated that its ingredients can be safely used to alleviate the symptoms of HF and improve myocardial contractile function, cardiac output, urinary production, and the renin-angiotensin-aldosterone system (RAAS)[14–17]. In 2004, QLQX capsules were approved by the China Food and Drug Administration (CFDA) for HF treatment. Nevertheless, the mechanism underlying the effects of QLQX remains not fully understood due to a lack of data from large-scale clinical trials. In 2009, we first reported that QLQX inhibits TNF-α while elevates IL-10 expression in rats with myocardial infarction (MI), ultimately leading to attenuation of cardiac remodeling[18]. This finding suggests the potential for better prevention and treatment of DCM patients by using QLQX to regulate the imbalance between pro- and anti-inflammatory cytokines.

In 2011, we designed the QLQX-DCM study, a randomized controlled trial (RCT), to evaluate the 12-month treatment effects of QLQX on the imbalance of pro- and anti-inflammatory cytokines in patients with DCM to assess the clinical benefits of QLQX compared to standard anti-HF treatment.

1 MATERIALS AND METHODS

1.1 Study Design and Participants

The QLQX-DCM study was a multicenter, double-blind, placebo-controlled, randomized trial conducted at 24 clinical research centers of tertiary hospitals across China. Patients with newly diagnosed virus-induced DCM were recruited from March 2012 to December 2015, and the cutoff time for follow-up was December 2017. Eligible participants included men and women aged 14 to 70 years. DCM was diagnosed according to the "1995 World Health Organization/International Society and Federation of Cardiology (WHO/ISFC) definition and classification of cardiomyopathies" and the "2013 European Society of Cardiology (ESC) statement on myocarditis"[19, 20]. In brief, the inclusion criteria were the presence of a left ventricular end-diastolic diameter (LVEDd) >5.5 cm in men or >5.0 cm in women, a left ventricular ejection fraction (LVEF) ≤45%, and a suspected or definite VMC at least 3 months before enrollment. The main exclusion criteria were idiopathic DCM or DCM caused by other reasons, including coronary artery disease, alcohol, pregnancy, etc. Patients with a potential survival time <1 year were also excluded. A full list of the inclusion and exclusion criteria is provided in table S2.

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of each study center. Written informed consent was obtained from all participants. The study was designed, implemented, and described following the CONSORT statement. The ClinicalTrials.gov number was NCT01293903.

1.2 Randomization and Blinding

The randomization sequence was generated at the coordinating center before the start of the trial. Participants were randomly assigned in a 1:1 ratio, with the use of a central interactive response system, to receive either the QLQX capsule or a placebo. During the random-sequence generation, stratification was performed according to the participating center. QLQX and placebo were packaged, labeled, stored, and distributed by staff who were not involved in the trial assessments. The appearance of the placebo capsules was identical to that of the QLQX capsules. All participants and investigators were blinded to the treatment assignment, and blinding was maintained until the end of the trial.

1.3 Intervention Assignment

After randomization, the participants received QLQX (1.2 g capsules three times daily, every 8 h) or placebo on the same schedule for 12 months. The study drugs were dispensed by a pharmacist at the hospitals. Participants returned for clinical visits every month after randomization to receive a new supply of capsules, adverse effects were assessed, and the primary and secondary endpoints were evaluated. Notably, all patients were also treated with standard anti-HF medicines in accordance with guidelines.

1.4 Study Procedures

Baseline clinical assessments and laboratory measurements were obtained during hospitalization to confirm the inclusion criteria. After that, laboratory measurements, including IFN-γ, IL-17, TNF-a, IL-4, IL-10, AHA, and NT-proBNP, were tested at the follow-up visits at the 1st, 3rd, 6th, and 12th month after randomization. Both plasma cytokine and AHA levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (PerkinElmer, USA and Watson Biotech, Inc., China, respectively), while NT-proBNP levels were measured using electrochemiluminescence immunoassay (ECLIA) kits (Roche Diagnostics GmbH, Germany). These 3 measurements were performed in the Laboratory of Cardiovascular Immunology, Institute of Cardiology, Wuhan Union Hospital, China.

Vital signs, other laboratory tests, electrocardiography (ECG) or Holter ECG, ultrasonic cardiogram (UCG), and chest X-ray were also obtained at enrollment and again

at the 12-month follow-up visit. Blood tests of complete blood count, liver and renal functions, cardiac enzymes, and even viral investigation were performed in local laboratories of the clinical centers.

Patients were instructed to report adverse events to the doctors as soon as possible. Doctors provided feedback immediately and advised patients on optimal treatment options or hospitalization. Adherence was assessed by counting the capsules that were returned by participants at each visit. Treatment adherence was defined as a ratio of >80% between the number of capsules taken and the number of capsules prescribed during the entire study period.

All the data were entered into an internet-based electronic data capture (EDC) system (http://www.chinamcdcm.com/), which was closed when the trial was finished.

1.5 Outcome Measures

The primary outcomes included changes in the LVEF, LVEDd, and plasma levels of inflammatory cytokines (IFN-γ, IL-17, IL-4, TNF-α, and IL-10) from baseline to 12 months after randomization. Secondary outcomes included all-cause mortality, hospital readmission due to HF, or worsening of DCM, which was defined as new or worsening symptoms or signs at 12 months. Events were adjudicated by an independent committee whose members were unaware of treatment assignments.

The exploratory endpoints were improvements in NYHA classification and reductions in the plasma levels of NT-proBNP. The safety endpoints included the incidence of adverse events, adverse drug reactions, and clinical laboratory tests.

1.6 Sample Size Calculations and Statistical Analysis

The sample size calculation was based on estimates, as there were no previous data on the use of QLQX in modulating inflammatory cytokine imbalances in patients with DCM. For clinical significance, the sample size was estimated using the improvement in LVEF, considering a 1:1 ratio of intervention to control participants. A calculated total of 266 participants were required to detect an increase in the mean LVEF of at least 10% in participants using QLQX compared to the placebo group, assuming a standard deviation of 25%, with 90% power and a two-sided alpha of 0.05. Considering the recruitment capacity of the trial centers and the feasibility of a conservative estimation, the target recruitment number was inflated to 345 to account for attrition. Additionally, this study provided an opportunity to examine the effects of QLQX on changes in LVEDd and cytokines (IFN-γ, IL-17, IL-4, TNF-α and IL-10) in this patient population. The sample size computations were based on a Bonferroni adjustment approach (i.e., each hypothesis test had a significance level of 0.0071). We estimated that with 345 participants, the trial would have 90% power to detect a 25% effect size with respect to each outcome.

Statistical analyses were performed on an intention-to-treat basis. Continuous variables are presented as mean ± SD or as the median (interquartile range, IQR), and categorical variables are presented as the number (percentage). The Chi-square test or Fisher's exact test (for categorical variables) and Student's *t* test or the Mann-Whitney *U* test (for continuous variables) were used where appropriate. The last observation carried forward (LOCF) method was used to impute data with dropouts. Bonferroni correction was used for adjustment to the significance level for each test, which used $\alpha 2=0.0071$ instead of $\alpha=0.05$. All the tests were two-sided. No interim analyses were performed.

The safety analyses were based on the treated population and included all patients who received at least one dose of QLQX or placebo. All the statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., USA).

2 RESULTS

A total of 782 patients were assessed for eligibility at 24 tertiary hospitals across China. Of these, 345 met the inclusion criteria and were randomly assigned to receive QLQX (n=171) or placebo (n=174), and 170 of these patients were enrolled in the VMC Registry Study[21]. At the end of the trial, 6 patients did not have discharge UCG measurements or laboratory data, including the plasma levels of inflammatory cytokines and NT-proBNP. The most common reasons for not returning to the clinic were distance to the clinic, referral care in another health facility, and finances. The screening, randomization, and follow-up of the trial participants are shown in fig. 1.

2.1 Baseline Characteristics

There were no significant differences between the QLQX group and the placebo group with regard to the characteristics of the participants at baseline (table 1). On average, the age was 49.8±13.4 years, and 73.3% were men. The LVEF was 33.2%±7.6%, and the LVEDd was 6.8±0.9 cm. Most patients present with moderate to severe HF, accompanied by high detection of circulating AHA and cardiotropic viruses (data not shown). Patients had a high rate of receiving RAAS inhibitors after randomization and during follow-up, adjusted according to patients' conditions. The mean adherence rate was 98.2% in the QLQX group and 98.3% in the placebo group (P>0.05).

2.2 Primary Outcomes

2.2.1 Effects of QLQX on Inflammatory Cytokines

As shown in fig. 2, QLQX treatment significantly decreased the plasma levels of the inflammatory cytokines (IFN-γ, IL-17, TNF-α, and IL-4) (all P<0.0001). Conversely, the levels of the anti-inflammatory cytokine IL-10 increased after QLQX treatment (P<0.0001). Although similar changes in the levels of these cytokines were also observed in the placebo group, the extent of these changes was greater in the QLQX group than in the placebo group (all P<0.0001).

Using the per-protocol (PP) population and the intention-to-treat (ITT) population to assess the data, the results of both analyses were concordant (table S3 and S4).

2.2.2 Effects of QLQX Treatment on LVEF and LVEDd

The effects of QLQX treatment on LVEF and LVEDd are

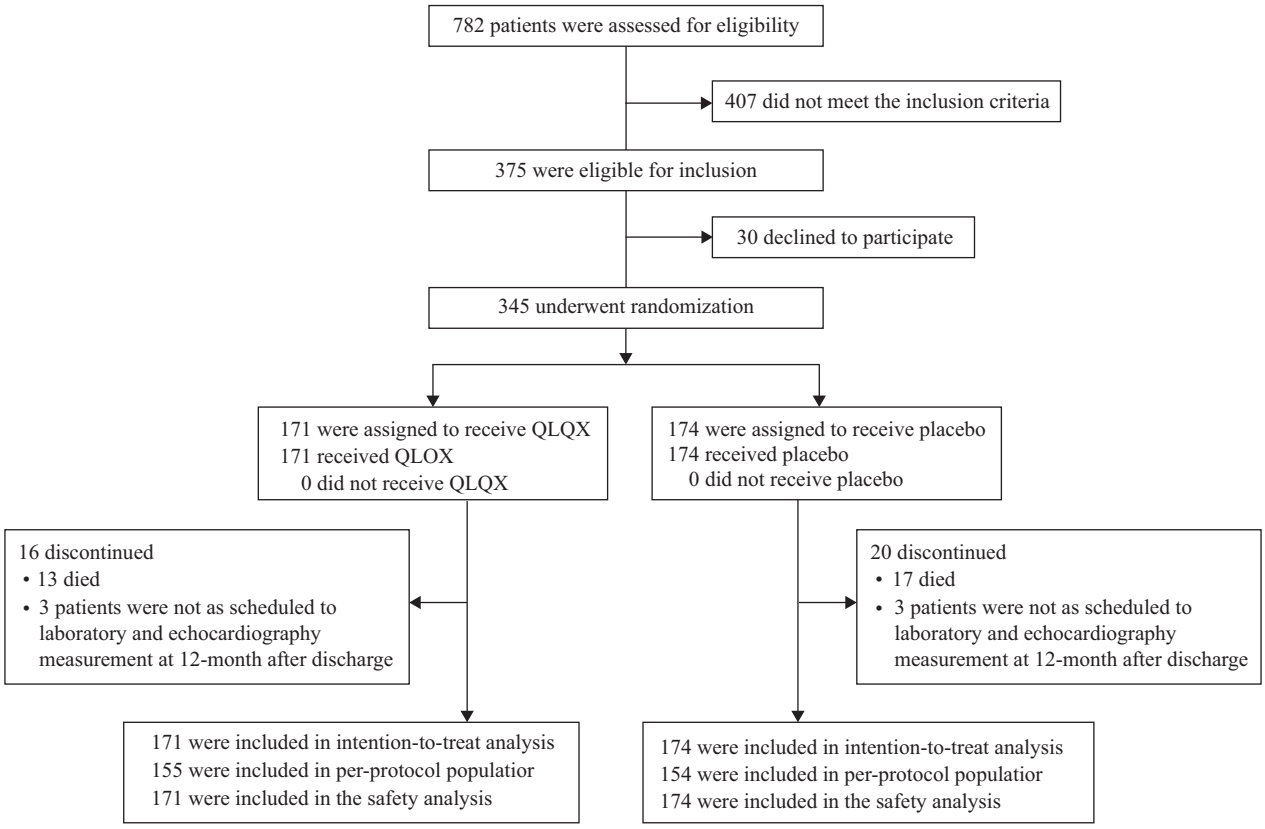

Fig. 1 Study flow diagram showing patient screening, randomization, and follow-up

presented in table 2. After the 12-month treatment, the LVEF increased from baseline in both groups (all $P<0.0001$), with a greater change in the QLQX group than in the control group (ΔLVEF: 9.46%±7.31% *vs*. 7.7%±6.71%, $P=0.0209$), and the percentage of patients with improved LVEF in the QLQX group was also higher than that in the placebo group (67.25% *vs.* 45.4%, $P=0.0001$). In addition, the LVEDd was also significantly reduced in both groups ($P<0.0001$). However, there were no differences in the level of change (ΔLVEDd: –0.59±0.46 cm *vs*. –0.56±0.63 cm, $P=0.5951$) or the percentage of improved patients between the QLQX group and the placebo group (55.6% *vs*. 48.9%, $P=0.2126$). These results were consistent with those of the PP analysis (table S5).

2.3 Effects of QLQX Treatment on NT-proBNP

At the end of the 12-month treatment, the plasma levels of NT-proBNP in patients taking QLQX were decreased compared with those at baseline ($P<0.0001$), as were those in the placebo group ($P<0.0001$). However, QLQX treatment led to a greater decrease than the placebo group ($P=0.0258$). After defining a decrease of at least 30% in NT-proBNP levels as treatment effectiveness over 12 months[14], it was found that QLQX treatment was effective in 68.4% of patients taking QLQX, while the placebo was effective in 50.0% of patients ($P=0.0005$) (table 3).

2.4 Effects of QLQX Treatment on NYHA

The improvement in the NYHA functional classification is shown in fig. 3. Despite a similar decrease in the NYHA functional classification in both groups (3.5% *vs*. 4.6%), compared with baseline, 59.1% of patients in the QLQX group ($P<0.0001$) and 35.1% in the placebo group ($P<0.0001$) were improved by at least one class of NYHA, and the difference between these two groups was statistically significant at month 12 of the study ($P<0.0001$).

2.5 Secondary Outcomes: Effects of QLQX Treatment on All-cause Mortality and Rehospitalization

At the end of the study, the total all-cause mortality, heart transplantation and rehospitalization rates were 8.7%, 2.03% and 35.65%, respectively. Notably, compared to the placebo group, 12 months of QLQX treatment led to a reduction in all-cause mortality (by 2.17%), heart transplantation (by 2.87%), the rehospitalization rate (by 2.28%), and the composite outcomes of mortality and transplantation (by 5.03%) in DCM patients, although there was no significant difference between the two groups (table 4).

2.6 Adverse Effects

No severe adverse events were reported in either group at the 12-month follow-up. Nevertheless, 5.26% of patients in the QLQX group had gastrointestinal discomfort and/or digoxin overdose in the blood, compared with 5.75% in the placebo group ($P=0.8438$). It was noted that the digoxin overdose disappeared when patients stopped taking digoxin for a few days. No participant discontinued participation in the study due to an adverse event (table 5).

3 DISCUSSION

This study demonstrated that QLQX treatment, which is based on standard anti-HF medications, significantly

Table 1 Baseline characteristics of the intention-to-treat population

Characteristics	Entire (*n*=345)	QLQX (*n*=171)	Placebo (*n*=174)	*P* values
Demographics				
Age (years)	49.8±13.4	50.1±13.6	49.4±13.3	0.6500
Men	253 (73.3%)	121 (70.8%)	132 (75.9%)	0.2840
With DCM family history	6 (1.7%)	2 (1.2%)	4 (2.3%)	0.4371
Smoking	135 (39.1%)	64 (37.4%)	71 (40.8%)	0.5204
Drinking	88 (25.5%)	45 (26.3%)	43 (24.7%)	0.7327
NYHA classification Ⅲ–Ⅳ	226 (65.51%)	118 (69.01%)	108 (62.07%)	0.1753
Heart rate (beats/min)	80.9±15.2	80.9±14.5	80.8±15.8	0.9390
Blood pressures				
SBP (mmHg)	117.5±17.6	117.1±16.0	117.8±19.0	0.7038
DBP (mmHg)	75.7±13.4	75.7±12.9	75.4±13.8	0.8352
ECG/Holter ECG				
LBBB	18 (5.22%)	7 (4.1%)	11 (6.3%)	0.4690
AVB	45 (13.04%)	25 (14.6%)	20 (11.5%)	0.3887
VPC (beats/min)	79 (22.9%)	36 (21.1%)	43 (24.7%)	0.4186
Atrial fibrillation	5 (1.5%)	3 (1.8%)	2 (1.2%)	0.6830
ST changes	162 (47.0%)	79 (46.2%)	83 (47.7%)	0.7798
Pacemaker	3 (0.87%)	1 (0.6%)	2 (1.2%)	1.0000
UCG measurements				
LVEF (%)	33.2±7.6	32.7±7.5	33.7±7.9	0.2326
LVEDd (cm)	6.8±0.9	6.8±0.9	6.7±1.1	0.8561
Laboratory parameters				
WBC ($\times10^9$/L)	7.3±2.2	7.2±2.1	7.5±2.2	0.2293
ALT (U)	29 (19, 45)	29.0 (18.0, 46.0)	26.0 (20.0, 44.0)	0.9778
BUN (mmol/L)	6.3 (5.3, 8.4)	6.4 (5.3, 8.6)	6.2 (5.0, 6.2)	0.2824
Serum creatinine (μmol/L)	83.5 (70.7, 98.0)	82 (68.7, 98.1)	82.1 (70.8, 96.1)	0.8689
Cardiac enzymes				
CK-MB (ng/mL)	6.2 (2, 13.6)	5.8 (2.1, 16)	4.8 (1, 13.6)	0.0973
cTN I (ng/mL)	0 (0, 0.2)	0.02 (0, 0.3)	0.02 (0, 0.13)	0.1797
NT-proBNP (ng/mL)	2096.8 (825.6, 4452)	2058 (860.2, 4680)	2177 (810.7, 4193.5)	0.7046
Pharmacological treatment				
ACEI/ARB	290 (84.06%)	144 (84.2%)	146 (83.9%)	0.9388
β-blockers	294 (85.22%)	147 (86.0%)	147 (84.5%)	0.6982
Spironolactone	282 (81.74%)	143 (83.63%)	139 (79.89%)	0.3686
Digoxin	163 (47.25%)	83 (48.54%)	80 (45.98%)	0.6338
Diuretics	214 (62.03%)	106 (61.99%)	108 (62.07%)	0.9877
Nitrates	98 (28.41%)	51 (29.82%)	47 (27.01%)	0.5624

The values are mean±SD, *n* (%) or medians (IQRs). The *P* value refers to the difference between the QLQX group and the placebo group, and a *P* value <0.05 was considered to indicate statistical significance.

ACEI: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers; AVB: atrioventricular block; BUN: blood urine nitrogen; CK-MB: creatine kinase-MB; cTN I: cardiac troponin I; DBP: diastolic blood pressure; ECG: electrocardiography; LBBB: left bundle branch block; LVEDd: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; SBP: systolic blood pressure; UCG: echocardiography; VPC: ventricular premature beats

alleviates immune imbalance in patients with DCM who have progressed from myocarditis by reducing the levels of the proinflammatory cytokines (TNF-α, IFN-γ, IL-17, and IL-4) while simultaneously increasing the levels of the anti-inflammatory cytokine IL-10. This potential regulatory role may be closely linked to the improvement in cardiac function observed in patients treated with QLQX for 12 months. Furthermore, although not statistically significant, compared with placebo, QLQX treatment reduced 12-month all-cause mortality by 2.17%, heart transplantation rate by 2.87%, and rehospitalization rate by 2.28%.

In recent years, immune inflammation has been gradually recognized as an underlying mechanism in the progression of chronic heart failure (CHF)[22–24]. Evidence for this inflammatory hypothesis mostly comes from experimental and clinical studies on DCM, especially those caused by viral infection[3–4]. Elevated levels of circulating and myocardial proinflammatory cytokines, including (TNF-α, IFN-γ, IL-17, IL-6, IL-1β and IL-4), have been demonstrated to be critical for cardiac impairment in DCM model animals[25–28]. However, whether increased cytokine serum levels in patients with DCM or CHF are a cause or a result of HF remains controversial, as several multicenter anti-TNF-α trials have reported discouraging results[13, 29, 30], despite some small-scale immunosuppression studies reporting cardiac improvements in patients with DCM[31]. In 2019, the publication of a prespecified subanalysis of the CANTOS trial provided new and encouraging evidence for the inflammatory mechanism in HF. Canakinumab,

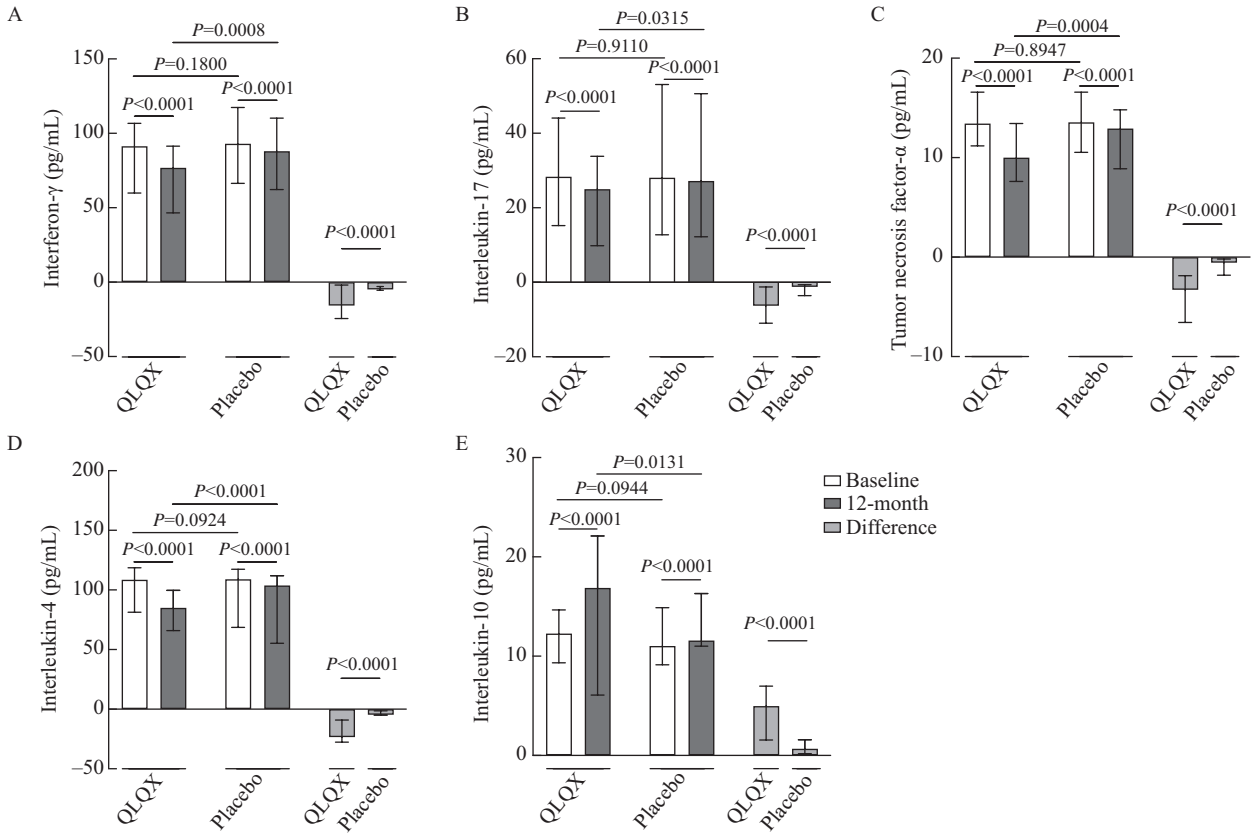

Fig. 2 Effects of QLQX *versus* placebo on changes in immune-inflammatory cytokines in the intention-to-treat population
A: interferon-γ; B: interleukin-17; C: tumor necrosis factor-α; D: IL-4; E: IL-10. The values are the medians (interquartile ranges). *P* values for differences between groups are shown.

Table 2 Effects of QLQX *versus* placebo on changes in LVEF and LVEDd in the intention-to-treat population

Parameters	QLQX (*n*=171)	Placebo (*n*=174)	*P* values
LVEF (%)			
Baseline	32.67±7.39	33.67±7.85	0.2231
12th month	42.13±10.15	41.38±10.11	0.4916
Difference from baseline (ΔLVEF)	9.46±7.31	7.7±6.71	0.0209
P	<0.0001	<0.0001	
LVEDd (cm)			
Baseline	6.8±0.9	6.78±0.91	0.8556
12th month	6.21±0.91	6.22±1.05	0.8973
Difference from baseline (ΔLVEDd)	–0.59±0.46	–0.56±0.63	0.5951
P	<0.0001	<0.0001	

The values are mean±SD or *n* (%). *P* values for differences between groups are shown in the rightmost columns (Student's *t* tests). *P* values on the left are differences from baseline to 12 months within groups (paired *t* test). LVEDd: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction

a therapeutic anti-IL-1β mAb, significantly reduced HF-related hospitalization and mortality in HF patients with previous MI and C-reactive protein (CRP) elevation[32]. Furthermore, a 5-year follow-up study on 57 DCM patients who underwent endomyocardial biopsy (EMB) revealed that higher levels of IL-6, TNF-α, adiponectin or hs-CRP are associated with more severe HF and lower survival rates[33]. Recently, the VMC Registry Study showed that sustained high levels of plasma IL-4 and IL-17 are predictive factors for the progression from VMC to DCM[21]. These emerging findings suggest that correcting inflammatory cytokine imbalances may benefit patients with HF. On the other hand, as cytokines may play multiple roles, the relatively high incidence of fatal infections or sepsis following canakinumab treatment in atherosclerosis patients implies that the overinhibition of a pro-inflammatory cytokine would lead to another immune dysfunction[34]. This might also be an important reason for the failure of anti-TNF-α trials. Targeting the imbalance of pro- and anti-inflammatory cytokines may provide a new treatment path for patients with HF or DCM.

QLQX is a TCM for HF treatment. In 2014, it was recommended as an important adjuvant medicine in the Chinese guidelines for the diagnosis and treatment of CHF[14, 35]. In this large, multicenter, double-blind RCT, we demonstrated the immunoregulatory function of QLQX in inflammation in VMC-progressed DCM patients, together with the significant improvements in LVEF, NT-proBNP, LVEDd and NYHA classification, meeting the prespecified primary endpoints. Consistent with previous reports in CHF patients, the serum levels of TNF-α, IFN-γ, IL-17, and IL-4 were elevated in all DCM patients at baseline. Both QLQX and placebo treatments, in conjunction with standard anti-HF medications, led to a decrease in the levels of these 4 proinflammatory cytokines and an increase in the level of the anti-inflammatory cytokine IL-10. The level of change in the QLQX group was greater than that in the placebo group. These results indicate that QLQX

Table 3 The favorable effect of QLQX treatment on NT-proBNP

	QLQX (*n*=171)	Placebo (*n*=174)	*P* values
NT-proBNP (ng/mL)			
Baseline	2058 (860.2–4680.0)	2177 (810.7–4193.5)	0.7046
12 months	792.3 (307.1–2118.0)	1074.7 (269.1–2432.0)	0.4865
Difference from baseline	–959.4 (–2444.1 to –291.3)	–705.2 (–1745.5 to –148.4)	0.0258
P	<0.0001	<0.0001	
12-month change from baseline			0.0005
Effective (decreased ≥30%)	117 (68.4%)	87 (50.0%)	
Ineffective (decreased <30%)	54 (31.6%)	87 (50.0%)	

P values for differences between groups are shown in the rightmost columns (Mann-Whitney *U* tests). *P* values on the left are differences from baseline to 12 months within groups (Wilcoxon signed-rank tests). NT-proBNP: N-terminal pro-B-type natriuretic peptide

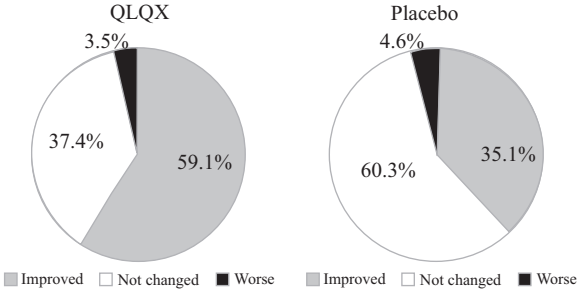

Fig. 3 Improvement in the NYHA functional classification following QLQX treatment
QLQX: Qiliqiangxin; NYHA: New York Heart Association

plays a role in regulating the imbalance between pro- and anti-inflammatory cytokines. In addition, they confirmed the anti-inflammatory properties of ACEI/ARB, which are associated with the inhibition of T-cell activation through blocking the endogenous renin-angiotensin system (RAS) of T cells[36], the downregulation of Th1 and Th2 cell-related chemokines[37], the promotion of regulatory CD4+Foxp3+ T cells (Tregs), and the suppression of Th1/Th17 cells[38]. Subsequently, in 2009, Li *et al* discovered that QLQX inhibited the expression of myocardial angiotensin Ⅱ in HF rats[39]. In 2012, Zou *et al* reported that QLQX can suppress the angiotensin Ⅱ type 1 (AT1) receptor and activate ErbB receptors to ameliorate cardiac remodeling and dysfunction in pressure-overloaded mice by inhibiting the ratio of TNF-α to the anti-inflammatory factor insulin-like growth factor-1 (IGF-1), attenuating cardiomyocyte apoptosis and autophagy, and promoting cardiomyocyte proliferation[40]. Furthermore, recent studies revealed that among the proinflammatory cytokines examined in this study, both Th1-related IFN-γ and Th17-related IL-17 also contribute to cardiomyocyte death[41–43], IL-4 drives the progression of myocarditis to DCM[44], and IL-6 mediates STAT3 signaling to participate in myocardial apoptosis in DCM mice[45]. Taken together with our previous study showing that QLQX regulates TNF-α and IL-10 in MI rats[18], this RCT demonstrated that the more significant amelioration of inflammatory cytokines, accompanied by greater improvements in cardiac function than in LVEDd in the QLQX group, suggested that QLQX has additional beneficial effects in DCM patients beyond its partial suppression of the RAS. The effects of QLQX on cardiac function in DCM patients may be closely linked to its regulatory role in correcting the imbalance of these pro- and anti-inflammatory cytokines.

Although 12 months of standard anti-HF treatment in the placebo group resulted in an all-cause mortality rate of 9.77% and a rehospitalization rate of 36.78%, which were lower than those reported in the Taiwan Society of Cardiology Heart Failure with reduced Ejection Fraction (TSOC-HFrEF) Registry (15.9% and 38.5%, respectively)[46] but slightly higher than those reported in the European Society of Cardiology Heart Failure Long-Term (ESC-HF-LT) Registry (8.8% and

Table 4 12-month clinical outcome analysis for treatment in the intention-to-treat population

	Entire (*n*=345)	QLQX (*n*=171)	Placebo (*n*=174)	*P* values
All-cause mortality	30 (8.7%)	13 (7.6%)	17 (9.77%)	0.4749
Heart Transplantation	7 (2.03%)	1 (0.58%)	6 (3.45%)	0.1213
Rehospitalization	123 (35.65%)	59 (34.5%)	64 (36.78%)	0.6586
All-cause mortality or heart transportation	37 (10.7%)	14 (8.19%)	23 (13.22%)	0.1310

The values are *n* (%). *P* values for differences between groups are shown in the rightmost columns (Chi-square test or Fisher's exact test, as appropriate).

Table 5 Adverse events from any cause in the intention-to-treat population

Averse events	Entire (*n*=345)	QLQX (*n*=171)	Placebo (*n*=174)	*P* values
Gastrointestinal discomforts	12 (3.48%)	5 (2.92%)	7 (4.02%)	0.7705
Digoxin overdose	4 (1.16%)	2 (1.17%)	2 (1.17%)	1.0000
Gastrointestinal discomforts and digoxin overdose	3 (0.87%)	2 (1.17%)	1 (0.57%)	0.6206
Total	19 (5.51%)	9 (5.26%)	10 (5.75%)	1.0000

The values are *n* (%). *P* values for differences between groups are shown in the rightmost columns (Fisher's exact test).
No severe adverse events were reported in either group. Similar frequencies were detected in the two treatment groups. No crossover occurred.

31.9%, respectively)[47] for patients with HFrEF during the same period, the addition of QLQX further reduced the mortality rate and rehospitalization rate by 2.17% and 2.28%, respectively, indicating a superior outcome compared to that of the ESC-HF-LT Registry. However, several limitations of the study may account for the absence of statistically significant differences in clinical outcomes, as well as in LVEDd, between the QLQX and placebo groups. The limited sample size and short one-year study period were insufficient to statistically discern the declining trend of clinical outcomes and LVEDd observed at 12th month. A longer follow-up period and larger sample size may statistically resolve the emerging trend. The majority of patients enrolled in this trial presented with moderate to severe HF, which might also be a factor influencing the efficacy of QLQX at 12 months. In addition, new anti-HF drugs, namely the angiotensin receptor-neprilysin inhibitor (ARNI) and the sodium-glucose cotransporter 2 inhibitor (SGLT2i), were not recommended for patients with HF at the time of this study. Further studies are needed to provide stronger evidence for the effects of QLQX on patients with DCM and its associated immunoregulatory mechanisms, and more studies should incorporate the use of ARNI and SGLT2i, as recommended by the new guidelines[48–50].

Finally, it should be noted that there were no severe QLQX-related adverse events during the course of the study, with the exception of a few patients who reported gastrointestinal discomfort, and most of them continued the study to complete. Elevated serum digoxin concentrations were also found but were rare in both groups. Patients with elevated serum digoxin concentrations stopped taking digoxin for several days until the blood concentration returned to normal before continuing the study without digoxin.

In summary, QLQX is a safe and effective TCM for treating DCM. QLQX can improve the cardiac function of patients with DCM secondary to VMC, probably by regulating the imbalance of pro- and anti-inflammatory cytokines. Combination treatment with QLQX and standard anti-HF medicines may lead to further benefits in patients with DCM. More studies in larger cohorts are needed to assess the association between clinical effects and the immunoregulatory role of QLQX in patients with DCM resulting from myocarditis.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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