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Randomised Controlled Trial into the role of ramipril in fibrosis reduction in RHD: The RamiRHeD trial protocol

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ABSTRACT

Introduction Rheumatic heart disease (RHD) is a major burden in developing countries home to 80% of all people living with the disease where it causes most of the cardiovascular morbidity and mortality in children and young adults. Chronic inflammation and fibrosis of heart valve tissue due to chronic inflammation in RHD will cause calcification and thickening of the impacted heart valves, especially the mitral valve. This fibrogenesis is enhanced by the production of Angiotensin II by increasing TGF-β expression and latter, the binding of IL-33 which is known to have anti-hyperthropic and anti-fibrotic effects to sST2. Its binding to the non-natural ligand of sST2 will worsen the fibrosis. Therefore, we hypothesized that Angiotensin-converting enzyme inhibitor (ACEI) will improve rheumatic mitral valve stenosis.

Methods and analysis This is a single-centre, double-blind, placebo-controlled, pre-post test design, randomised clinical trial. Patients with mitral stenosis valvular dysfunction due to rheumatic disease planned for cardiac valve replacement operation will be given Ramipril 5 mg or placebo for a minimum of 12 weeks before the surgery. Expression of ST2 in the mitral valve is considered to be representative for cardiac fibrosis. Mitral valve tissue will be stained using the immunohistochemistry method using ST2. While plasma ST2 will be measured by ELISA. This study is conducted in the Department of Cardiology and Vascular Medicine, Universitas Indonesia, National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia from June 27th, 2019.

Ethical Approval and Dissemination Ethics of this study has been approved by the ethical committee of National Cardiovascular Center Harapan Kita with ethical code: LB.02.01/VII/286/KEP.009/2018. This study has been registered in clinicaltrials.gov with identifier code of NCT03991910.

Stregths and limitations of the study

- This study is the first study that analysed the valve fibrosis using immunohistochemistry of ST2 in rheumatic mitral stenosis patients.
- This study proposed novel treatment targeting the valve fibrosis reduction in rheumatic mitral stenosis patients.
- This research will help low-to-middle income countries to economically treat rheumatic mitral stenosis patients by attenuating the progression of mitral valve fibrosis.
- The limitation of this study is that the mitral valve surgery schedule can be variable and not fix (could be sooner or later than the expected date) so the time of ramipril or placebo consumptions could be differ for each patients.
- No standard control of the non-fibrotic valve, because all patients have rheumatic mitral stenosis and the mitral valves only could be obtained during the mitral valve surgery, but

plasma ST2 concentration and the echocardiographic parameters could help to define the severity of the valve fibrosis before treatments.
- Patients recruitments takes time longer than usual due to COVID-19 pandemic.

INTRODUCTION

Rheumatic heart disease (RHD) is a major burden in developing countries, home to 80% of all people living with the disease where it causes most of the cardiovascular morbidity and mortality in children and young adults. More than 15.6 million people globally suffer from RHD, and 233.000 die prematurely of the disease every year. According to the 2010 Global Burden of Disease Study, the number of disability-adjusted life years lost to RHD was as high as 10.1 million per year worldwide.[1] In Indonesia, the rate of rheumatic heart disease with mitral valve stenosis was about 94 cases each year.[2] Diagnosis and treatment during the advanced stage of the disease are very costly and challenging especially in the developing countries. Early diagnosis and prompt treatment of the RHD population can be of great help for the targeted treatment of the disease.[3] Rheumatic mitral valve stenosis is the main presentation of RHD that leads to significant morbidity and mortality. Mitral stenosis usually develops as a result of persistent or recurrent valvulitis with bicommissural fusion.[4] fibrogenesis are stimulated and activated by various stimuli such as cytokines, connective tissue growth factors, and activators. Previous studies suggest that RHD is an autoimmune disease that is associated with cytokine activation.[4] Inflammatory cytokines are key regulators of immune processes. Chronic inflammation causes damage to the valvular tissue. Many studies investigated the potential biomarkers to evaluate fibrosis and chronic inflammation process in RHD patients, and ST2 is one of the sensitive marker for detecting cardiac fibrosis, including the fibrosis proccess in RHD [4–6]

ST2 is a member of the interleukin (IL)-1 receptor family discovered in a classical translational science fashion and exists in two forms, a transmembrane receptor (ST2L) as well as a soluble decoy receptor (sST2).[7] A member of the interleukin (IL)-1 receptor family, ST2 is a biomarker of mechanical stress, up-regulated in isolated cardiomyocytes exposed to mechanical strain; derangement of ST2 signalling leads to a phenotype consistent with myocardial remodelling and in patients with heart failure, sST2 levels strongly correlate with the severity of heart failure, independently forecasting risk additive to NT-proBNP and other biomarkers. Both sST2 and ST2L are induced in cardiomyocytes and fibroblasts exposed to biomechanical stress. Biomechanical stress and fibrosis will enhance the valve thickening in RHD.[8] Clarifying the understanding of the role played by ST2 in cardiovascular disease, IL-33 has been shown to have anti-hypertrophic and anti-fibrotic effects in the heart, transduced by ST2L.[7] Calcification and thickening of the mitral valves were enhanced by the production of Angiotensin II. Angiotensin II induce the upregulation of Transforming growht factor β (TGF-β) and latter the binding of IL-33 to sST2 and not to the natural ligand (ST2L). Binding of IL-33 to sST2 will cause fibrogenesis evenmore. Thus, ACEI are hypothized to attenuate this viscious cycle through the inhibition of Angiotensin II and consequently increase Bradykinin that furtherly inhibit fibrosis through the negative regulation of angiotensin II activity in Mitogen Activator Protein Kinase (MAPK) pathways through the suppression of the Ca2+ response and the Na+ transport.[9,10]

ACEI are often used in preventing and treating heart failure due to regurgitant valve disease. The majority of patients with symptomatic RHD have significant mitral stenosis (MS) and are denied ACEI therapy, because of the fear of hypotension in the presence of fixed obstruction.[11] ACEI are first-line therapy in heart failure, and their symptomatic and survival benefits extend beyond afterload reduction. Reduction in fibrosis and anti-proliferative and neurohumoral effects contribute to the ACEI effect that is not reproduced by pure vasodilators. ACEI was well tolerated in symptomatic RHD associated with significant

mitral stenosis and preserved left the ventricular systolic function.[11] Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. The only established therapeutic options for rheumatic mitral stenosis were balloon mitral valvuloplasty and mitral valve surgery. More economical therapeutic option that targeted the inhibition of fibrogenesis and improve the mitral valve fibrosis is needed especially in low to middle income countries. Valvular anti-inflammatory and anti-fibrosis medical therapy to suppress the progression of the disease is needed in rheumatic mitral stenosis patients. ACEI (Enalapril) was well tolerated in symptomatic RHD associated with significant aortic and/or mitral stenosis and preserved left ventricular systolic function.[11]

To date, there is no treatment of rheumatic mitral stenosis that focus on the main pathogenesis, the valvular dibrosis. For this reason, new strategies and therapies are needed to prevent the progression of RHD.[4] Neutralizing inflammatory cytokines or antagonizing their receptor function has been considered as a useful therapeutic strategy to treat autoimmune diseases.[4] In this respect, new therapies targeting ST 2 and their receptors as studied in some autoimmune diseases may promise a new approach for patients with RHD. ACE inhibitors are agents with anti-fibrotic effects. This study therefore aims to investigate the effect of the ACE inhibitor Ramipril in suppressing the expression of ST2 in the cardiac mitral valve in patients with RHD (Figure 1).

METHODS AND ANALYSIS

Study Designs

This is a single-centre, double-blind, placebo-controlled, pre-post test design, randomised clinical trial. Patients with mitral stenosis valvular dysfunction due to rheumatic process planned for cardiac valve replacement will be treated with Ramipril 5 mg or placebo for minimum 12 weeks (3 months) before the surgery. ST2 will be checked as fibrosis marker (Figure 2). This is conducted and still recruiting, in the National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia from June 27th, 2019.

Study Population

Patients with rheumatic mitral valve stenosis (RMS) who undergo cardiac valve replacement operation in National Cardiovascular Center Harapan Kita (NCCHK), Jakarta, Indonesia will be screened for eligibility criteria of this study. Inclusion criteria of this study are: patients with RMS or combined valve disease aged more than 18 years who undergo cardiac valve repair or replacement operation with or without tricuspid valve repair. Patients with systolic blood pressure (SBP) ≥ 100 mmHg and diastolic blood pressure (DBP) ≥ 60 mmHg. Exclusion criteria of this study are: Patients with congenital heart disease, patients with non mitral valve surgery, patients with coronary artery bypass surgery and patients who refuse to provide informed consent. Further exclusion criteria are adults aged 65 years or over, pregnant women, and patients with autoimmune disease, patients with persistent hypotension (SBP < 100 mm Hg), severe aortic stenosis (aortic valve orifice < 0.75 cm2), chronic renal dysfunction with serum creatinine > 2.5 mg/ dL, or known ACEI intolerance. Participants who meet the criteria and are willing to join the RamiRHeD trial will be informed in detail about the study participation and will be required to sign the informed consent.

Outcomes

The primary outcomes of this study are the ST2 expression in mitral valve tissue and the secondary outcomea are: soluble plasma ST2, clinical signs and symptoms, and the other echocardiography and laboratory test results.

Sample Size and Randomisation

This is a pioneer study for analysing effects of Ramipril 5 mg toward ST2 expresion in mitral valve tissue in human. Previous study that use ST2 human tissue is a study from Marzullo et at in 2016[12] that use carotid tissue from carotid endarterectomy, with sample size of 41 consecutive patients. Because our study will use human tissue sample, we approached the sample size calculation using multistage non-finite population method, using this specified precision estimation formula[13]: $N = (Z\delta)/E$, with N= sample size; $Z_{0.95}$= 1.96; δ N(0,1) = 1; and E= 0.05 for 0.95 confidence interval. So we calculate the sample and found that 1.65(1)/0.05= 33 samples.

According to the sample size of the previous study that analyse ST in human tissue and a sample size formula that commonly used in in-vivo study, we decide to use a sample size of 30 for each arm, and with the addition of drop out rate of 10%, become total of 66 for 2 arms. The number includes a 10% dropout and withdrawal in each group. Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.

Research technique

Mitral valve surgery (MVS) is performed with Mitral Valve Replacement. The echocardiography established the diagnosis of Rheumatic Mitral Valve Disease. Rheumatic valve disease was diagnosed with World Heart Federation Criteria (2012) for RHD[14]. The reference measurement for valve area is a planimetry by two-dimensional echocardiography. Doppler technique assessed for the mean mitral gradient. The degree of mitral regurgitation was evaluated according to Seller's classification on left ventriculography in a 30° right anterior oblique view. In cases of missing data, substitution measurements will be used as previously described: Doppler half time pressure for valve area and colour Doppler for mitral regurgitation.[14]

Patients will be diagnosed with experienced cardiologists, surgery desicion based on the decision of a multidisciplinary team, echocardiography will be performed by the cardiologist who is specialized in echocardiography. Blood samples will be drawn by the experienced nurses in pathology clinic laboratory, ST2 plasma and tissue will be collected with the biomedical anlalysts, interview of study participants will be done by the trained assessors. Study instruments involve questionnaires, laboratory tests, echocardiography, and biochemical tests. Study instruments will use same technique, same tools, same brands, and same place for data collection of each study participant.

Intervention

Daily administration of capsules containing Ramipril 5 mg or placebo orally will be provided for study's participants. Participants will remain under the care of the cardiologist treating team. Routine medications of each patient will be continued. Capsules containing Ramipril 5 mg or placebo will be given for a minimum of 3 months before the operation schedule.

Withdrawal and Drop out

Participants will be informed that they are able to withdraw from the study at any time and will sign a reference as proof. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry. Drop out criteria will be participants who are lossed to follow up, participants with severe adverse events, mortality due to any cause.

Sample Collection and Measurements

Clinical signs and symptoms will be documented before and after the study. Blood samples will be collected twice: before the intervention and a day before the mitral valve surgery. The routine blood sample includes haemoglobin, platelet count, leucocyte count, erythrocyte sedimentation rate (ESR), C-Reactive Protein (CRP). Total cholesterol, random blood glucose, HbA1C, urea, creatinine, serum electrolytes, NTproBNP, plasma ST2 will be determined. Echocardiography before the intervention and before surgery will be performed. Mitral valve tissue expression of ST2 will be measured using the immunohistochemistry method. Plasma ST2 will be measured using Enzyme Link Immunoabsorbent Assay (ELISA) kit for human ST2/IL33R antibody with catalogue number DST200 from R&D. This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ST2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ST2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ST2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of ST2 bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

Mitral valve tissue will be collected during mitral valve replacement surgery and will be saved in a sterile container filled with formalin 10%. Mitral valves tissue will be stained using immunohistochemistry (IHC) method to assess ST2 expression. Chemical fixation uses cross-linking chemicals such as paraformaldehyde and glutaraldehyde to preserve the cellular structure. Once the tissue is harvested, the fixation begins. Following the fixation, a tissue block is made by placing the sample in hot parafilm, placed into a mould, allowed to cool and harden, and thin tissue sections can then be made. De-cloaking methods include heat and pressure treatment, enzyme digest, and microwaving. Following the de-cloaking, the parafilm on the slides is removed by baking, and the IHC staining process can begin. The source of the primary antibody that will be used is from the monoclonal type of ST2 antibody. The secondary antibody will be conjugated by biotin. Blocking buffer will include BSA. The chromogen that will be used is 3,3'-Diaminobenzidine (DAB). An oxidized-DAB that is

catalysed with Horseradish peroxidase (HRP) then will form a brown precipitate, so the ST2 expression can be visualised using light microscope. The tissue will then be counterstained using hematoxylin-Eosin staining, so the non-ST2-expressing-cells can be visualised in bluish colour A negative control will use Hematoxylin-eosin staining only. Measurements of cells which express ST2 will be done under the microscope. Date, tissue type, antibody dilution, tissue treatment, and magnification of the microscope will be documented. Cells which express ST2 will be counted by more than one professionals.

Statistical Analysis

Continuous variables are expressed as mean±SD and categorical variables as percentages. The $\chi 2$ test will be used to see the relationship between dichotomous variables and the Student t-test for continuous variables. Single variable correlation analysis and multivariable linear regression analysis will be performed. A P value <0.05 was considered statistically significant. The analyses were performed with the use of SPSS for Windows.

Ethics and Dissemination

Ethics of this study has been approved by the ethical committee of National Cardiovascular Center Harapan Kita (NCCHK), Jakarta, Indonesia, with ethical code: LB.02.01/VII/286/KEP.009/2018. This study has been registered in clinicaltrials.gov with identifier code of NCT03991910.

DISCUSSION

This study is planning to recruiting rheumatic mitral valve patients to be randomised for getting capsules either containing Ramipril 5 mg or placebo. Rheumatic mitral stenosis is the main presentation of RHD that leads to significant morbidity and mortality. Mitral stenosis usually develops as a result of persistent or recurrent valvulitis with bicommissural fusion. Previous studies suggest that RHD is an autoimmune disease that is associated with cytokine activities. Inflammatory cytokines are key regulators of immune processes.[4] Imunologic reaction caused by the autoreactive antibody continuously caused the chornic inflammation and valvular fiibrosis, which can be detected by the increase of sST2 as the emerging biomarker for cardiac fibrosis.[10,15,16]

ST2 binds IL-33. It is a pro-inflammatory IL-1 family cytokine with intracellular and extracellular activities. IL-33 is expressed in smooth muscle and airway epithelia.[17] ST2 is upregulated by inflammatory stimulation of some cells, such as: keratinocytes, and dermal fibroblasts and by mechanical strain in cardiac fibroblasts.[17,18] The soluble ST2 isoform is elevated in the serum under inflammatory conditions such as allergic asthma, sepsis, trauma, dengue fever, and pulmonary disease.[19–22] Serum ST2 elevation is also associated with multiple aspects of heart failure including aortic stenosis, congestive cardiomyopathy, and risk of heart failure and death.[23–27] In this study, plasma ST2 is considered as the inflammatory and fibrosis biomarker for the rheumatic mitral stenosis patients. Because plasma ST2 also can increase in various conditions unrelated to cardiac fibrosis, this study also measures the ST2 expression in mitral valve tissue. Plasma ST2 describes the amount of

ST2 in the circulation, whereas mitral valve cells which express ST2 describe the ST2 that bind to the domain receptor.

ACEI are commonly administered as the treatment of heart failure due to valvular regurgitation. Its use in MS is still debatable because of its hypotensive effect. A prior study assessing the safety of ACEI in MS patients showed that ACEI (specifically Enalapril in that study) was well tolerated and safe for untill dose of 10 mg bid.[11] ACEI is pressumed to have vasodilatory effect in obstructive lesions, It will decrease systemic vascular resistance through arterial vasodilatation, thus will increase the transvalvular gradient. Its anti-remodelling effect is also well established and its long term use also has been proven to improve Left Ventricular Ejection Fraction (LVEF) in patients with systolic dysfunction.[28] Because the prior study[11] demonstrated the efficacy and the potential benefits of ACEI on improving outcomes in MS patients, this study wants to reconfirm and investigate the possible pathomechanism of those improvements. This study will assess the effect of Ramipril 5 mg as the cardiac antifibrosis treatment in severe MS RHD patients. Their plasma ST2 concentration will be measured and compared. Plasma ST2 concentration also will be compared before and after several months of consuming Ramipril 5 mg. There will be no healthy control for this study because of the ethical limitation in the acquisition of mitral valve tissue. Mitral valve tissue will be acquired during the mitral valve surgery. Expression of ST2 in mitral valve tissue will then be calculated semi-quantitatively and compared with the plasma ST2 results. It is hypothesized that Ramipril will supress the expression of ST2 in the cardiac mitral valve in patients with RHD.

Beside plasma ST2 and ST2 expression in mitral valve tissue, this study also compares the pre-post effects of Ramipril 5 mg versus placebo to the NT-proBNP concentrations echocardiography strain parameters, and also clinical outcomes. Clinical signs and symptoms and echocardiography parameters were evaluated in some studies of mitral valve stenosis, and showed that they were positively correlated with the NT-proBNP concentration.[29,30] This study will also compare the NT-proBNP concentration of patients receiving Ramipril and placebo. NT-proBNP concentrations also can be corellated with the ST2 concentration and expression.

Authors contributions

Conception and design of the work was initiated by AMA, BS, AS, BR, and BD. AMA, BD, ES, FT contributed to the acquisition, analysis, and interpretation of data for the work. Manuscript was drafted by AMA, BD, and ES. AMA, PD, AW, and MJC critically revised the manuscript. Author and co-authors gave final approval and agree to be accountable for all aspects of work, ensuring integrity and accuracy.

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Competing interests statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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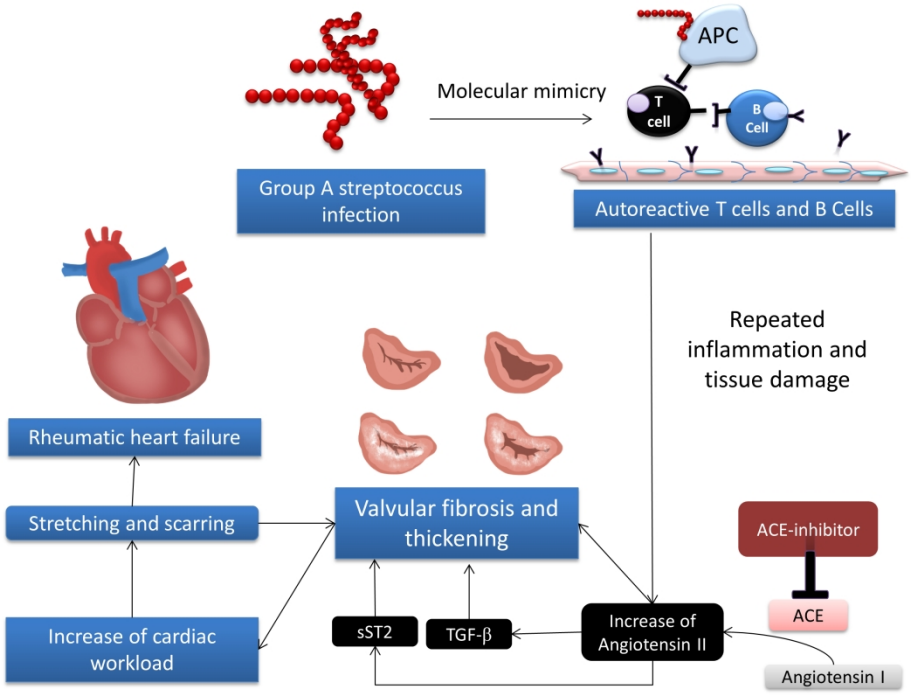

Figure 1 Hypothesis
Molecular mimicry is a defense mechanism of group A streptococcus to avoid immune cells. This mechanism allows immune cells to generate autoimmunity against protein the lining of endothelial cells and causing chronic inflammation and valvular damage. Continous proccess of chronic inflammation leads to valvular thickening and fibrosis, which is mediated by the Angiotensin II. Angiotensin II increase TGF-β expression and cause IL-33 to bind with sST2, and subsequently cause damage and fibrosis to the valvular tissue evenmore, which later will ended with rheumatic heart failure. ACEI is hypothized to counteract these processes by decreasing Angiotensin Iconversion from Angiotensin I.

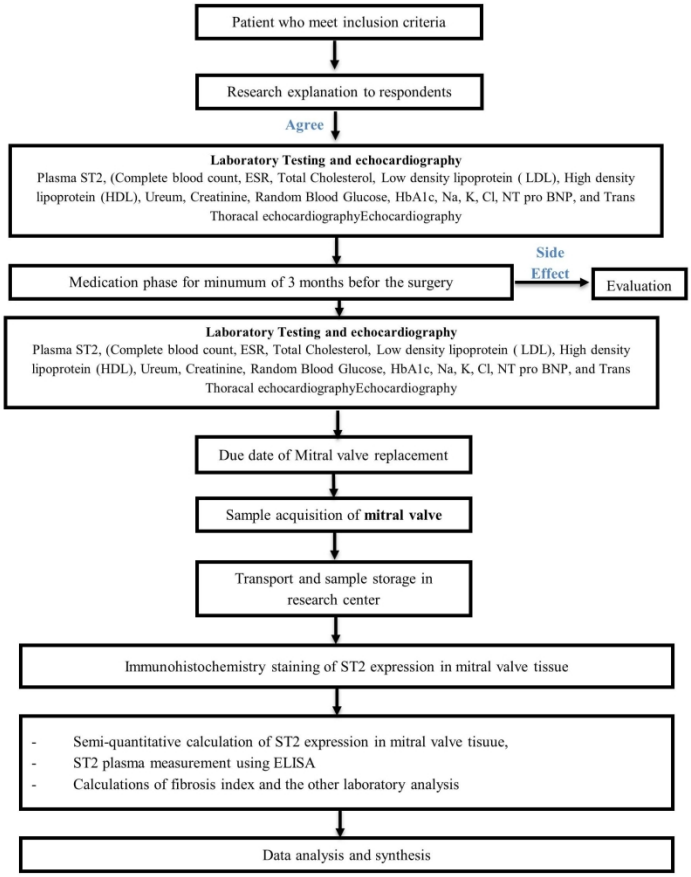

Figure 2. Research Flow-chart

Figure 2. Research Flow-chart

190x274mm (284 x 284 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Randomised Controlled Trial into the role of ramipril in fibrosis reduction in RHD: The RamiRHeD trial protocol
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Clinicaltrials.gov NCT03991910

2b All items from the World Health Organization Trial Registration Data Set

Register	ClinicalTrials.gov
Main ID	NCT03991910
Protocol ID	RamiRHeD
Date of Registration	19/06/2019
Prospective Registration	Yes
Primary sponsor	Harapan Kita National Cardiovascular Center/Indonesia University
Public title	The Effect of Ramipril in Suppressing ST2 Expression in Rheumatic Mitral Stenosis Patients
Scientific title	The Effect of Ramipril in Suppressing Gene Expression of Fibrosis in Cardiac Mitral Stenosis in Patients With Rheumatic Heart Disease
Date of first enrloment	June 27, 2019
Target sample size	66
Recruitment status	Recruiting
Study type	Randomised clinical trial
Study design	Allocation: Randomized. Intervention model: Parallel Assignment. Primary purpose: Treatment. Masking: Double (Participant, Investigator).
Phase	Phase 3
Countries of Recruitment	Indonesia
Health condition	ACE inhibitor Fibrosis; heart Mitral stenosis Rheumatic heart disease Rheumatic mitral stenosis
Intervention (s)	Drug: placebos Drug: Ramipril 5 mg oral capsule
Primary outcome	ST2 expression in mitral valve tissue
Secondary outcome	ST2 plasma level NT-proBNP concentration (pg/ml) Ejection fraction TAPSE (tricuspid annular plane systolic excursion NYHA class

Protocol version 3 Date and version identifier

Released: 19-06-2019

Funding 4 Sources and types of financial, material, and other support

No specific fundings outside our institution (Harapan Kita National Cardiovascular Center, Indonesia)

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors

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Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands

Role: Ade M. Ambari conceived the study. Ade M. Ambari, Pieter A. Doevendans, Maarten .J.M. Cramer, Budhi Setianto, Anwar Santoso, Basuni Radi, Bambang Dwiputra initiated the study design and conceptual framework. Ade M. Ambari, Bambang Dwiputra contribute in patients' recruitment and assessment,writing and editing. Eliana Susilowati contribute to the biomedical methods, sample collection, writing and editing. Fadilla Tulrahmi conducting the primary statistical analysis, randomisation, and sample size calculation. Ade M. Ambari, Pieter A. Doevendans, Maarten .J.M. Cramer, Annemiek Wind contribute to writing, protocol editing, language editing. All authors contributed to refinement of the study protocol and approved the final manuscript.

5b Name and contact information for the trial sponsor

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Contact name: Ade Meidian Ambari
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Telephone: 0215681111
Email: dr_ade_meidian@yahoo.co.id

5c Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

5d Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

Introduction

Principal Investigators
Design and conduct of RCTRHDMS
Preparation of protocol and revisions
Organising steering committee meetings
Publication of study reports
Members of TMC [*Trial Management Committee*]

Steering committee (SC)
Agreement of final protocol
All lead investigators will be steering committee members
Reviewing progress of study and if necessary agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study.

Trial Management Committee (TMC)
(Principle [*sic*] investigator, Research Physician, Administrator)
Study planning
Organisation of steering committee meetings
Provide annual risk report to ethics committee
report serious adverse events (SAE) to medical committee and ethics committee
Responsible for trial master file
Budget administration and contractual issues with individual centres
Advice for lead investigators
Audit of 6 monthly feedback forms and decide when site visit to occur.
Assistance with international review, board/independent ethics committee applications
Data verification
Randomisation
Organisation of central serum sample collection

Data Manager
Maintenance of trial IT system and data entry
Data verification

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Rheumatic heart disease (RHD) is a major burden in developing countries home to 80% of all people living with the disease where it causes most of the cardiovascular morbidity and mortality in children and young adults. Chronic inflammation and fibrosis of heart valve tissue due to chronic inflammation in RHD will cause calcification and thickening of the impacted heart valves, especially the mitral valve. This fibrogenesis is enhanced by the production of Angiotensin II by increasing TGF-β expression and latter, the binding of IL-33 which is known to have anti-hyperthropic and anti-fibrotic effects to sST2. Its binding to the non-natural ligand of sST2 will worsen the fibrosis. Therefore, we hypothesized that Angiotensin-converting enzyme inhibitor (ACEI) will improve rheumatic mitral valve stenosis.

Existing knowledge: Angiotensin-converting enzyme inhibitors (ACEI) are first-line therapy in cardiac failure, and their symptomatic and survival benefits extend beyond afterload reduction. Reduction in fibrosis and anti-proliferative and neurohumoral effects contribute to the ACEI effect that is not reproduced by pure vasodilators. ACEI was well tolerated in symptomatic RHD associated with significant mitral stenosis and preserved left the ventricular systolic function. New therapies targeting ST 2 and their receptors as studied in some autoimmune diseases may promise a new approach for patients with RHD. We are assessing the effect of Ramipril in suppressing fibrotic protein expression in mitral valve (measured with ST2 expression) of patients with RHD in the National Cardiac Center Harapan Kita hospital Jakarta Indonesia.

Dose selection: Prevoious study (SCOPE trial) showed that ACEI (Enalapril) was well tolerated in symptomatic RHD associated with significant aortic and/or mitral stenosis and preserved left ventricular systolic function until dose of 10 mg bid. This study use Ramipril 5 mg as it is more commonly used in Indonesia.

Need for a trial: Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. In developing countries, percutaneous balloon mitral valvuloplasty and mitral valve surgery are the therapeutic options for rheumatic mitral stenosis. Both of these treatments involve enormous expenses; it is the public health cost burden for developing countries. Valvular anti-inflammatory and anti-fibrosis medical therapy to suppress the progression of the disease is needed in rheumatic mitral stenosis patients.

	6b	Explanation for choice of comparators Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. This study is divided in 2 arms. The first arm will be given Ramipril 5 mg as the intervention, while the second arm, will be given placebo as the comparator. The other individualized treatments for rheumatic valvular disease was still given in both armsas indicated.
Objectives	7	Specific objectives or hypotheses The investigators hypotized that administration of Ramipril 5 mg for 3 months will reduce expression of ST2 as fibrosis biomarkers, in the cardiac mitral valve of patients with Rheumatic Heart Disease with mitral stenosis.
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) This is a single-centre, double-blind, placebo-controlled, pre-post test design, randomised clinical trial. Patients with mitral stenosis valvular dysfunction due to rheumatic process planned for cardiac valve replacement were given Ramipril 5 mg or placebo for minimum 12 weeks before the surgery. ST2 was checked as fibrosis marker. This study will be conducted in the Department of Cardiology and Vascular Medicine, University Indonesia, National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia from June, 27th 2019.

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Study setting: in a national academic hospital. Patients come from various regions in one country (Indonesia).
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) • Inclusion criteria of this study are: Patients with mitral valve stenosis or a combination • aged more than 18 years • undergo cardiac valve replacement operation with or without a tricuspid valve repair, • patients with systolic blood pressure (SBP) ≥ 100 mmHg and diastolic blood pressure (DBP) ≥ 60 mmHg • passed in medication phase without side effect minimum 4 weeks until operation schedule

Interventions

11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered

Arms and Interventions

Arms	Interventions
Placebo Comparator: control control patients will be given a placebo	Drug: Placebos the control group will be given placebo inside a capsule, so study participant won't be able to know the drug and doses inside the capsule (for masking)
Experimental: treatment Ramipril 5 mg treatment group	Drug: Ramipril 5Mg Oral Capsule the treatment group will be given Ramipril 5 mg inside a capsule, so study participant won't be able to know the drug and doses inside the capsule (for masking)

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)

Participants will be informed that they are able to withdraw from the study at any time and will sign a reference as proof. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry. Drop out criteria will be Participants who are lost to follow up, participants with severe adverse events, mortality due to any cause.

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)

Patients' adherence will be monitored with the drug return. Patients will be contacted via phone number to be reminded for routine administration. Patients are provided with the investigator contact if needed something to ask or to report adverse events or side effects (if any). Patients will be asked to visit the hospital where this study is enrolled to meet the investigator monthly. Each month, capsule return will be counted and patient will also be evaluated for the symptoms and vital signs.

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial

Rescue medication: stop the drug. Patient will be admitted to hospital and undergone treatments according to the adverse effects.

Prohibited concomitant medication: Angiotensin receptor blocker.

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended

Primary Outcome Measures :

- ST2 plasma level

plasma level of ST2 measured by ELISA

Secondary Outcome Measures :

- ST2 expression in mitral valve tissue

expression of ST2 in mitral valve tissue, using immunohistochemistry method

- NT-proBNP concentration (pg/ml)

concentration of NT-proBNP, plasma markers for cardiac dysfunction.

- ejection fraction

echocardiography parameter

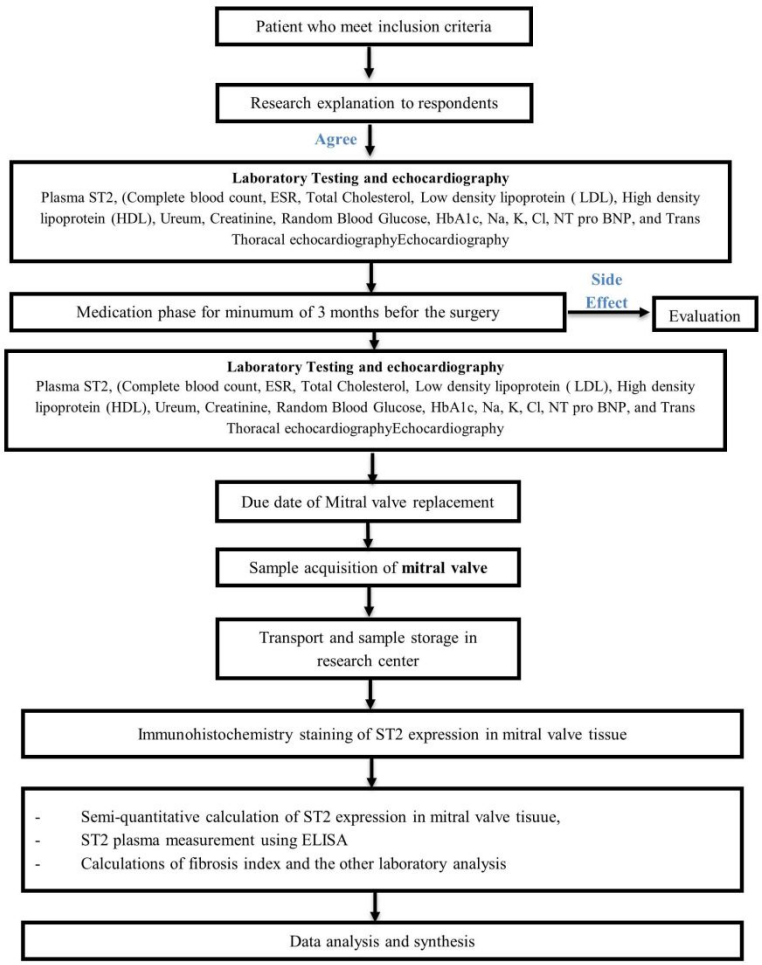
- TAPSE (tricuspid annular plane systolic excursion)

echocardiography parameter to asses right ventricular function

- NYHA class

related symptoms will be graded in class I to IV according to NYHA.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)



Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations This is a pioneer study for analysing effects of Ramipril 5 mg toward ST2 expresion in mitral valve tissue in human. Previous study that use ST2 human tissue is a study from Marzullo et at in 2016[12] that use carotid tissue from carotid endarterectomy, with sample size of 41 consecutive patients. Because our study will use human tissue sample, we approached the sample size calculation using multistage non-finite population method, using this specified precision estimation formula[13]: $N = (Z\delta)/E$, with N= sample size; $Z_{0.95}$= 1.96; δ N(0,1) = 1; and E= 0.05 for 0.95 confidence interval. So we calculate the sample and found that 1.65(1)/0.05= 33 samples. According to the sample size of the previous study that analyse ST in human tissue and a sample size formula that commonly used in in-vivo study, we decide to use a sample size of 30 for each arm, and with the addition of drop out rate of 10%, become total of 66 for 2 arms. The number includes a 10% dropout and withdrawal in each group. Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Patient recruitment will involves several cardiologists and cardiothoracic surgeons. All cardiologists and cardiothiracic surgeons in the hospital will be informed about this study, and to inform back if their patient suffered from Mitral valve stenosis. Technical meetings will be held and the profesionals related to this study will be invited.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Allocation sequence generation are from online, web-based sequence generator system. Participants will be enrolled with staff member responsible for patients enrollment.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Trial participants, investigators, analysts, care providers, will be blinded. Reserach assisstants whose role are to follow-up, evaluate and monitor the patients condition will not be blinded, so the drug could be stopped easily if there is any adverse effect from the treatment group.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Under the circumstances where actual treatment is absolutely necessary for further management of the patient, unblinding is permissible.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Data collections will involve profesional in each filed of data collection. For example: patients will be diagnosed with experienced cardiologists, surgery desicion based on the decision of a multidisciplinary team, echocardiography will be performed by the cardiologist who is specialized in echocardiography. Blood samples will be drawn by the experienced nurses in pathology clinic laboratory, ST2 plasma and tissue will be collected with the biomedical anlalysts, interview of study participants will be done by the trained assessors which. Study instruments involve questionnaires, laboratory tests, echocardiography, and biochemical tests. Study instruments will use same technique, same tools, same brands, and same place for data collection of each study participant.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Participants will be informed that they are able to withdraw from the study at any time and will sign a reference as proof. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Participant files will be stored in numerical order and stored in a secure and accessible place and manner. Participants data will be copied in softfile and have back-up data.

Statistical methods 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol

Variable/Outcome	Hypothesis	Outcome Measure	Methods of Analysis
1) <u>Primary</u>	Participants adherence of Ramipril 5 mg for 3-6 months or placebo 3-6 months will be 80%	Remaining drug in the participants is not more than 20% of the study drug given, to evaluate the adherence.	Manual counting, univariate analysis
a) ST2 expression in mitral valve tissue	Reduction occurr	Mitral valve tissue ST2 measured using percentage of-cell-expressing ST2 using immunohistochemistry method	T-test
2) <u>Secondary</u>			
a) ST2 plasma level	Reduction occurr	Plasma ST2 concentration will be measured with the concentration calculated according to the absorbance in ELISA technique	T-test
b) NT-proBNP concentration	Reduction occur	NT-proBNP concentration is measured in clinical pathology laboratorium using ELISA.	T-test
c) Ejection fraction(EF) and TAPSE	Improvement occurr	Measure was performed using same echocargiography pre and post test, documented in percentage.	T-test
d) NYHA class	Reduction occurr	NYHA class will be measured accoding to the clinical signs and symptoms classified in NYHA	Chi-square test

20b Methods for any additional analyses (eg, subgroup and adjusted analyses)

Continuous variables were expressed as mean±SD and categorical variables as percentages. The $\chi 2$ test was used to see the relationship between dichotomous variables and the Student t-test for continuous variables. Single variable correlation analysis and multivariable linear regression analysis will be performed. A P value <0.05 was considered statistically significant. The analyses were performed with the use of SPSS for Windows.

20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

This study will be analysed in per-protocol fashion. Handling missing data will use multiple imputation method. Analysis of the primary endpoint will be based on a log-rank test and, therefore, not affected by patient withdrawals (as they will be censored) provided that dropping out is unrelated to prognosis. The effect that any missing data might have on results will be assessed via sensitivity analysis of augmented data sets. Dropouts (essentially, participants who withdraw consent for continued follow-up) will be included in the analysis by modern imputation methods for missing data. After the imputations are completed, all of the data (complete and imputed) will be combined and the analysis performed for each imputed-and-completed dataset. Rubin's method of multiple imputation will be used to estimate treatment effect.

Methods: Monitoring

Data monitoring

21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

A Data Monitoring Committee (DMC) has been established. The DMC is independent of the study sponsor. During the period of recruitment to the study, interim analyses will be supplied, in strict confidence, to the DMC, together with any other analyses that the committee may request.

21b Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial

An interim-analysis is performed on the primary endpoint when 80% of patients have been randomised and mitral valves have been obtained. The interim-analysis is performed by a statistician in this study member. Stopping decision (if needed) based on the desicion of the primary investigators and will be reported to the ethics commitee.

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Adverse events that happen before the patient started to receive study intervention will not be reported as it is not related to the study drug. All adverse events occurring after the patient receiving study intervention until the end of the study will be recorded. Serious adverse event (SAE) related to this study treatment will be reported to the institutional review board and ethical committee. Serious adverse event including: life-threatening condition with immediate risk of death, severe or permanent disability, prolonged hospitalisation, or a sgnificant hazard determined by the data safety monitoring board. SAE that is believed by the investigator and medical committee to be causally related to the study drug will be reported. SAE occuring a month after the subject is discontinued from the study will not be reported unless the investigators believed that the event have been caused by the study drug. The causal effects will be determined according to the temporal relationship, clinical course, previous medical conditions and concomitant medications.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor Ethic committee will audit the trial conduct

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval This protocol and the template informed consent forms contained in Appendix I has been reviewed and approved by the IRBs/ECs [*institutional review boards/ethical committees*] with respect to scientific content and compliance with applicable research and human subjects regulations. The protocol, site-specific informed consent forms is in local language. Participant education and recruitment materials, and other requested documents and any subsequent modifications also will be reviewed and approved by the ethical review bodies (IRBs/ECs).

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. If such amandement happens, it will request an approval of the Ethics Committee/IRB [*institutional review board*] prior to implementation.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) The member of investigators will explain and obtain informed consent.
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Additional biological samples will be obtained to be stored for use in future studies. It will be stored in research center inside this hospital with adequate and certified samples handling. A materials consent will be obtained to the collection of the plasma speciments.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial All study-related information will be stored securely in the principal investigator's locker with limited access. All participants data will be identified by a coded ID number to maintain the confidentiality. Laboratory speciments will be stored in research center with a safe storage and will be identified also with coded ID. Local database will be secured with password access systems and the access will be limited.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site All of the investigators disclose no conflict of interests.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Data management coordinating team will oversee the intra-study data sharing process. All principal investigators will be given access to the cleaned datasets. Principal investigators will have direct access to their own data sets and will have access to other sites data by request. To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Study participants are covered by compensation for negligent harm through the standard of this hospital. This will include cover for additional health care, if it has causal relationship with the study drug.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Investigators is not expected to report the data as individual report. All presentations and publications are expected to protect the integrity of the major objectives of the study; data that break the blind will not be presented prior to the release of the main results. Recommendation as to the timing of presentation of endpoint data which they might be presented will be given by the steering committee. Each paper abstract must be submitted to the appropriate subcommittee for review of its appropriateness and scientific merit prior to submission, the subcommittee may recomend changes to the authors and submit its recommendations to be approved by the steering committee. Publications of papers to workshops, symposia, volumes etc will be in the right of the principal investigators, and principal investigators could appoint and give permission for the other investigators or other party to present this paper. the study results will be released to the participating physicians, patients, and general medical community.
	31b	Authorship eligibility guidelines and any intended use of professional writers Topics suggested for presentation or publication will be circulated to the PIs [*Principal investigators*] of the CCCs [*Core Coordinating Centers*], the DCC [*Data Coordinating Center*], and research center in hospital. These groups are requested to suggest and justify names for authors to be reviewed by the PC [*Publications Committee*].
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code Data sharing statement: no later than 5 years after the collection of the 1-year post randomisation interviews, we will deliver a completely deidentified data set to an appropriate data archive for sharing purposes.

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates

RAHASIA 1

Lembar Informasi dan Persetujuan Pasien

Judul Penelitian :

The Effect of Ramipril in Suppressing Gene Expression of Fibrosis in Left Atrial Appendage in Cardiac Mitral Stenosis Rheumatic Heart Disease Patients

Latar Belakang dan Tujuan Penelitian

Penyakit jantung rematik merupakan beban penyakit utama di negara berkembang yang hampir 80% seluruh orang hidup dengan penyakit ini, dimana sebagai penyebab tingginya angka kematian dan kesakitan kardiovaskular pada anak dan remaja. Berdasarkan *Global Burden of Disease Study* (2010), jumlah pasien yang mengalami disabilitas karena penyakit jantung rematik sebanyak 10,1 juta per tahun di seluruh dunia.[1] *Rheumatic Mitral Valve Stenosis* (RMS) merupakan indikator utama penyakit jantung rematik yang dapat berdampak signifikan pada kematian dan kesakitan.

ST2 merupakan bagian reseptor IL-1 yang terdiri dari 2 bentuk, *a trans-membrane receptor* (ST2L) dan *soluble decoy receptor* (sST2).[4] Keduanya diinduksi di *cardiomyocyes* dan *fibroblast* yang terpapar tekanan biomekanik. Fungsi dari ST2 pada penyakit kardiovaskular, IL-33 telah terbukti memiliki efek anti-hipertrofik dan anti-fibrotik pada jantung, ditransduksi oleh ST2L.[4]

Angiotensin-Converting Enzyme Inhibitors (ACEI) sering digunakan untuk mencegah dan mengobati gagal jantung karena penyakit katup regurgitasi. Mayoritas pasien dengan penyakit jantung rematik simtomatik (RHD) memiliki mitral stenosis (MS) yang signifikan dan menolak terapi ACEI, hal ini disebabkan karena ditakutkan adanya hipotensi di kemudian hari dengan adanya obstruksi tetap.[5] ACEI pula dapat ditoleransi pada penyakit jantung rematik simtomatik berkaitan dengan mitral stenosis yang signifikan dan tetap mempertahankan fungsi sistolik ventrikel kiri.[5]

Efikasi pencegahan sekunder terbatas dalam mencegah progresivitas penyakit jantung rematik sehingga diperlukan adanya strategi dan terapi yang dibutuhkan untuk mencegah hal tersebut.[3] Terapi terbaru menargetkan ST2 dan reseptor seperti yang diteliti pada penyakit autoimun memungkingkan adanya pendekatan baru untuk pasien penyakit jantung rematik. ACEI merupakan agen dengan efek anti fibrosis. Oleh karena itu, peneliti ingin mengetahui efek Ramipril dalam memodulasi ekpresi gen fibrosis pada

jaringan katup dan appendiks atrium kiri pasien dengan penyakit jantung rematik di RS Jantung dan Pembuluh Darah Harapan Kita.

Penjelasan tentang Prosedur Pelaksanaan

Pasien dengan penyakit jantung rematik mitral stenosis yang akan dilakukan tindakan MVR/r akan diberikan Ramipril atau Placebo selama minimal 3 bulan dan maksimal 6 bulan yang akan dikonsumsi setiap hari sampai waktu untuk dilakukannya tindakan operasi.

Partisipasi sukarela/pengunduran diri

Partisipasi anda dalam penelitian ini bersifat sukarela, anda dapat menolak untuk berpartisipasi. Jika anda memutuskan untuk berpartisipasi, maka anda akan diberi lembar informasi ini untuk dipelajari isi dan tujuan penelitian. Anda dapat menyimpan lembar informasi ini dan anda akan diminta untuk mengisi dan menandatangani formulir persetujuan ini.

Anda dapat mengundurkan diri di awal saat anda membaca informasi dari penelitian ini dan tidak menyetujui untuk mengikuti penelitian tanpa harus memberikan alasan. Pengunduran diri anda tidak menimbulkan sanksi apapun dan anda tidak akan kehilangan manfaat yang akan menjadi hak anda.

Manfaat partisipasi

Secara pribadi anda dapat mengambil manfaat berpartisipasi dalam penelitian ini karena dapat membantu mengurangi gejala dan tanda penyakit jantung katup mitral rematik. Anda juga dapat memberikan manfaat bagi orang lain dalam pengembangan ilmu pengetahuan dan peningkatan kesehatan masyarakat secara luas.

Resiko dan Ketidaknyamanan

Konsekuensi dari partisipasi ini mengharuskan anda untuk minum obat maksimal selama 6 bulan setiap hari sebelum dilakukannya tindakan MVR/r. Partisipan akan melakukan pengambilan obat setiap bulannya ke tempat penelitian. Selain itu juga akan menjalani beberapa pemeriksaan penunjang medis seperti echocardiography dan

pengambilan darah yang akan dilakukan sebelum fase minum obat, dan setelah selesai minum obat.

Kerahasiaan

Semua data pada penelitian ini akan diambil tanpa memberikan identitas anda. Kerahasiaan data dan identitas Anda dilindungi oleh hukum dan atau peraturan yang berlaku, dan tidak akan diberitakan secara umum. Pada saat hasil diumumkan, identitas Anda akan tetap terjaga kerahasiaannya.

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Persetujuan Komite Etik Kedokteran

Penelitian ini diteliti dan disetujui oleh Komisi Etik Pusat Jantung Nasional Harapan Kita.

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Partisipan yang mengikuti penelitian ini akan diberikan biaya perjalanan/transportasi dari rumah ke tempat penelitian sebesar Rp. 100.000,- pada setiap bulan selama masa konsumsi obat. Biaya penelitian ditanggung oleh peneliti yang termasuk dalam paket penelitian.

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Jika anda merasa tidak nyaman, anda dapat memilih untuk tidak ikut serta dalam penelitian. Hal ini tidak akan mempengaruhi pelayanan rumah sakit terhadap anda di masa mendatang

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Jika Anda mempunyai pertanyaan-pertanyaan mengenai studi ini, Anda dapat menghubungi:

dr. Ade Meidian Ambari, SpJP(K)
No. telepon. 021 – 568 4085 ext 2209

Silahkan untuk tidak mendatangani formulir ini jika anda tdak mempunyai kesempatan untuk bertanya atau tidak menerima jawaban-jawaban yang memuaskan terhadap pertanyaan-pertanyaan anda.

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Dengan menandatangani formulir ini, saya menyetujui bahwa penelitian ini telah dijelaskan kepada saya dan semua pertanyaan saya telah dijawab dengan memuaskan. Saya juga mempunyai hak untuk dapat mengundurkan diri dari penelitian ini setiap saat. Dengan pengertian tersebut, saya dengan sukarela ikut serta dalam penelitian ini. Saya mengerti bahwa formulir ini akan disimpan bersama dengan data kesehatan saya dan saya akan mendapatkan *copy* dari formulir ini.

Nama Pasien : _____	Nama Wali : _____
Tanda tangan : _____	Tanda tangan : _____
Tanggal : ___/___/_____	Tanggal : ___/___/_____

Nama Dokter / Asisten : _____
Tanda tangan : _____
Tanggal : ___/___/_____

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Plasma speciments will be stored in biobank inside the research center in this hospital, and has been approved by the medical and ethical committee for the possible further researches.

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Randomised Controlled Trial into the role of ramipril in fibrosis reduction in Rheumatic Heart Disease: The RamiRHeD trial protocol

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ABSTRACT

Introduction Rheumatic heart disease (RHD) is a major burden in developing countries and accounts for 80% of all people living with the disease, where it causes most cardiovascular morbidity and mortality in children and young adults. Chronic inflammation and fibrosis of heart valve tissue due to chronic inflammation in RHD will cause calcification and thickening of the impacted heart valves, especially the mitral valve. This fibrogenesis is enhanced by the production of angiotensin II by increased TGF-β expression and later by the binding of IL-33, which is known to have anti-hypertrophic and anti-fibrotic effects, to soluble sST2. sST2 binding to this non-natural ligand worsens fibrosis. Therefore, we hypothesise that angiotensin-converting enzyme inhibitors (ACEIs) would improve rheumatic mitral valve stenosis.

Methods and analysis This is a single-centre, double-blind, placebo-controlled, randomised clinical trial with a pre-post test design. Patients with rheumatic mitral stenosis and valve dysfunction will be planned for cardiac valve replacement operation will be given ramipril 5 mg or placebo for a minimum of 12 weeks before the surgery. The expression of ST2 in the mitral valve is considered to be representative of cardiac fibrosis. Mitral valve tissue will be stained by immunohistochemistry to ST2. Plasma ST2 will be measured by ELISA. This study is conducted in the Department of Cardiology and Vascular Medicine, Universitas Indonesia, National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia, starting on June 27th, 2019. The performance and dissemination of this study were approved by the ethics committee of National Cardiovascular Center Harapan Kita with ethical code LB.02.01/VII/286/KEP.009/2018. This study has been registered at clinicaltrials.gov with the identifier code NCT03991910.

Strengths and limitations of the study

- A novel study that analysed the ST2 expression in rheumatic heart patients' mitral valves.
- This study proposed novel and affordable treatment targeting the rheumatic heart valve fibrosis reduction.
- This research will help low-to-middle-income countries treat rheumatic heart disease more economically.
- Flexible schedule of mitral valve surgery causes different time range of the intervention for each patient.
- No standard healthy control of the non-fibrotic valve, based on ethical consideration.

INTRODUCTION

Rheumatic heart disease (RHD) is a serious health problem in developing nations, where it affects 80% of the population and accounts for the majority of cardiovascular morbidity and mortality in children and young adults. RHD affects more than 15.6 million individuals worldwide, with 233,000 people dying prematurely each year. [1] In the past 5 years, approximately 471 rheumatic mitral stenosis patients were treated in our Centre. [2] Treatments provided for RHD in advanced stages are relatively expensive for developing nations; thus, early detection and targeted treatment can greatly aid.[3] Mitral valve stenosis is the main presentation of RHD, commonly developing as a result of persistent or recurrent valvulitis with bicommissural fusion.[4] Fibrogenesis is induced by various stimuli, such as cytokines, connective tissue growth factors, and activators. Previous studies suggest that RHD is an autoimmune disease that is associated with cytokine activation.[4] Inflammatory cytokines are key regulators of immune processes. Chronic inflammation causes damage to the valvular tissue. Many studies have investigated potential biomarkers to evaluate fibrosis and chronic inflammation processes in RHD patients, and ST2 is a sensitive marker for detecting cardiac fibrosis, including fibrosis progression in RHD.[4–6]

ST2 is a member of the interleukin (IL)-1 receptor family discovered in a classical translational science fashion, and it exists in two forms: a transmembrane receptor (ST2L) and a soluble decoy receptor (sST2).[7] As a member of the interleukin (IL)-1 receptor family, ST2 is a biomarker of mechanical stress that is up-regulated in isolated cardiomyocytes exposed to mechanical strain; derangement of ST2 signalling leads to a phenotype consistent with myocardial remodelling, and in patients with heart failure, sST2 levels strongly correlate with the severity of heart failure, independently forecasting risk on top of the risk from NT-proBNP and other biomarkers. Both sST2 and ST2L are induced in cardiomyocytes and fibroblasts exposed to biomechanical stress. Biomechanical stress and fibrosis will enhance valve thickening in RHD.[8] Clarifying the role played by ST2 in cardiovascular disease, IL-33 signalling through ST2L has been shown to have anti-hypertrophic and anti-fibrotic effects in the heart.[7] Calcification and thickening of the mitral valves are enhanced by the production of angiotensin II. Angiotensin II induces the upregulation of transforming growth factor β (TGF-β) and later the binding of IL-33 to sST2 instead of to its natural receptor ST2L. Binding of IL-33 to sST2 will cause fibrogenesis. Thus, ACEIs are hypothesised to attenuate this vicious cycle by inhibiting angiotensin II and consequently increasing bradykinin, which further inhibit fibrosis through the negative regulation of angiotensin II activity in mitogen-activated protein kinase (MAPK) pathways through the suppression of the Ca2+ response and Na+ transport.[9,10]

ACE inhibitors are frequently used to prevent and treat heart failure caused by regurgitant valve disease. Because of the risk of hypotension in the presence of a fixed obstruction, the majority of patients with symptomatic RHD have substantial mitral stenosis (MS) and refuse ACEI medication.[11] ACEI is the primary treatment for heart failure. The way ACEIs improve clinical symptoms and survival outcomes is to advance afterload reduction. Fibrosis attenuation and its anti-proliferative effects and neurohormonal effects are superior to those of pure vasodilators.[11] Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. The only established therapeutic options for rheumatic mitral stenosis are balloon mitral valvuloplasty and mitral valve surgery. More economical therapeutic options that target the inhibition of fibrogenesis and improve mitral valve fibrosis are needed, especially in low- to middle-income countries. Valvular anti-inflammatory and anti-fibrotic medical therapy to slow the progression of the disease is needed in rheumatic mitral stenosis patients. One ACEI (enalapril) was well tolerated in symptomatic RHD associated with significant aortic and/or mitral stenosis and preserved left ventricular systolic function.[11]

Currently, there is no treatment for rheumatic mitral stenosis that targets the main pathogenesis, valvular fibrosis. Therefore, novel approaches and therapies are needed to prevent RHD progression.[4] Neutralising inflammatory cytokines or antagonising their receptor function has been considered a useful therapeutic strategy to treat autoimmune diseases.[4] In this respect, new therapies targeting ST2 and its ligands, as studied in some autoimmune diseases, may be a new approach for patients with RHD. ACEIs are agents with anti-fibrotic effects. This study therefore aims to investigate the effect of the ACE inhibitor ramipril in suppressing the expression of ST2 in the cardiac mitral valve in patients with RHD (Figure 1).

METHODS AND ANALYSIS

Study Designs

This is a single-centre, double-blind, placebo-controlled, randomised clinical trial with a pre-post test design. Rheumatic mitral stenosis patients with valvular dysfunction who are scheduled for cardiac valve replacement will be treated with ramipril 5 mg or placebo for a minimum of 12 weeks (3 months) before the surgery. ST2 will be checked as a fibrosis marker (Figure 2). The study is still recruiting patients at the National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia, from June 27th, 2019.

Study Population

Patients with rheumatic mitral valve stenosis (RMS) who undergo cardiac valve replacement in the National Cardiovascular Center Harapan Kita (NCCHK), Jakarta, Indonesia, will be screened for eligibility. The inclusion criteria of this study are patients with RMS or combined valve disease aged more than 18 years who undergo cardiac valve replacement operation with or without tricuspid valve repair. Patients must also have systolic blood pressure (SBP) ≥ 100 mmHg and diastolic blood pressure (DBP) ≥ 60 mmHg. The exclusion criteria of this study are patients with congenital heart disease, non mitral valve surgery, coronary artery bypass surgery or refusal to provide informed consent. Further exclusion criteria are adults aged 65 years or over, pregnant women, and patients with autoimmune disease, persistent hypotension (SBP < 100 mmHg), severe aortic stenosis (aortic valve orifice < 0.75 cm2), chronic renal dysfunction with serum creatinine > 2.5 mg/dL, or known ACEI intolerance. Participants who meet the criteria and are willing to join the RamiRHeD trial will be informed in detail about the study and will be required to sign the informed consent.

Outcomes

The primary outcomes of this study are the ST2 expression in mitral valve tissue, and the secondary outcomes are soluble plasma ST2, clinical signs and symptoms, and other echocardiography and laboratory test results. Study participants will be followed up for cardiac and all-cause mortality outcomes until 1 year after the surgery.

Sample Size and Randomisation

This is a pioneering study analysing the effects of 5 mg ramipril on ST2 expression in mitral valve tissue in humans. A previous study that used ST2 human tissue was conducted by Marzullo et al in 2016[12]. The used carotid tissue from carotid endarterectomy, with a sample size of 41 consecutive patients. Because our study will use human tissue samples, we approached the sample size calculation using the multistage non-finite population method, using this specified precision estimation formula[13]: $N = (Z\delta)/E$, with N= sample size; $Z_{0.95}$= 1.96; δ N(0,1) = 1; and E= 0.05 for a 0.95 confidence interval. Therefore, we calculated a required sample of 1.65(1)/0.05= 33 samples.

According to the sample size of the previous study that analysed ST in human tissue and a sample size formula that is commonly used in *in vivo* studies, we decided to use a sample size of 30 for each arm, and with the addition of a drop out rate of 10%, this became total of 66 for the 2 arms.

The number includes a 10% dropout and withdrawal from each group. Randomisation will be done with an equal ratio of ramipril to placebo. A computerised sequence generator is used for randomisation. It will be linked with codes for placebo and treatment tablets provided by the manufacturer that was contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule will be identical between the two groups and will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.

Research technique

The mitral valve surgery (MVS) will be mitral valve replacement. Echocardiography will establish the diagnosis of rheumatic mitral valve disease. Rheumatic valve disease will be diagnosed with World Heart Federation Criteria (2012) for RHD[14]. The reference measurement for valve area is planimetry by two-dimensional echocardiography. The Doppler technique is used to assess the mean mitral gradient. Seller's classification on left ventriculography in a right anterior oblique view angle of 30° will be performed to evaluate the severity of mitral valve regurgitation. In cases of missing data, substitution measurements will be used as previously described: Doppler half time pressure for valve area and colour Doppler for mitral regurgitation.[14]

Patient classification and diagnosis of rheumatic mitral stenosis will be determined by qualified cardiologists, and the decision to perform mitral valve replacement surgery will be based on the consensus of the multidisciplinary team, consisting of cardiologists and cardiothoracic surgeons. Echocardiography will be performed by echocardiography-consultant cardiologists. Blood samples will be collected by trained nurses specialised in pathology clinic laboratory work. Biomedical analysts will be in charge of the analysis and collection of ST2 in plasma and mitral valves. Detailed interviews with the study participants will be done by a well-trained medical doctor. The data will come from questionnaires, laboratory tests, echocardiography, and biochemical tests. The study instruments will use the same technique, same tools, same brands, and same place for data collection from each study participant.

Pre-existing atrial fibrillation, left atrial size, concomitant rheumatic valve disease, NYHA class, and other clinical data and echocardiographic data will be documented before and after surgery and will be analysed by multivariate analysis.

Intervention

Daily capsules containing 5 mg ramipril or placebo to be taken orally will be provided for the study participants. An initial dose of 2.5 mg of ramipril will be given to the patients in the intervention group. If there are no significant adverse effects documented in the first 2 weeks after the initial dose, 5 mg of ramipril will be given in the subsequent weeks until 5 days before mitral valve replacement surgery. Participants will remain under the care of the treating cardiologist team. The routine medications of each patient will be continued. Capsules containing 5 mg ramipril or placebo will be given for a minimum of 3 months, up until 5 days before the mitral valve replacement.

Withdrawal and Drop out

Participants will be informed that they are able to withdraw from the study at any time and will sign a form stating this. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accordance with the consent obtained at trial entry. Drop-out criteria will be loss to follow-up, severe adverse events, and mortality due to any cause.

Sample Collection and Measurements

Clinical signs and symptoms will be documented before and after the study. Blood samples will be collected twice: before the intervention and one day before the mitral valve surgery. The routine blood analysis will include haemoglobin, platelet count, leucocyte count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Total cholesterol, random blood glucose, HbA1C, urea, creatinine, serum electrolytes, NTproBNP, and plasma ST2 will be determined. Echocardiography before the intervention and before surgery will be performed. Mitral valve tissue expression of ST2 will be measured by immunohistochemistry. Plasma ST2 will be measured using an enzyme-linked immunoabsorbent assay (ELISA) kit with the human ST2/IL33R antibody (R&D Systems, catalogue number DST200). This assay uses the technique of the quantitative sandwich enzyme immunoassay. A monoclonal antibody specific for human ST2 is pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any ST2 present is bound by the immobilised antibody. Unbound substances are washed away and then, an enzyme-linked polyclonal antibody specific for human ST2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells, and colour develops in proportion to the amount of ST2 bound in the initial step. After the colour development is stopped, the colour intensity is measured.

Mitral valve and papillary muscle tissue will be collected during mitral valve replacement surgery and will be saved in a sterile container filled with 10% formalin. ST2 expression will be observed using immunohistochemistry (IHC). Cross-linking chemicals, such as paraformaldehyde and glutaraldehyde, will be used to preserve the cellular structure. The fixation begins when the tissue is harvested. Tissue blocking is performed afterwards by placing the tissue sample in hot parafilm, after which it is put into a mould until hard. Following fixation, tissue sections are obtained using a microtome. Decloaking methods consisting of heat and pressure treatment, enzyme digestion, and microwaving are done afterwards. Following decloaking, the parafilm on the slides is removed by baking, and then the IHC staining process can be started. The primary antibody is a monoclonal ST2 antibody. The secondary antibody

is conjugated by biotin. The blocking buffer includes BSA. The chromogen that will be used is 3,3'-diaminobenzidine (DAB). DAB oxidation is catalysed by horseradish peroxidase (HRP), after which it forms a brown precipitate, so ST2 expression can be visualised under a light microscope. The tissue will then be counterstained using haematoxylin-eosin staining, so the non-ST2-expressing cells can be visualised in bluish colour. A negative control will use haematoxylin-eosin staining only. Measurements of cells that express ST2 will be performed under a microscope. The date, tissue type, antibody dilution, tissue treatment, and magnification of the microscope will be documented. ST2-expressing cells will be counted by more than one professional.

Statistical Analysis

Continuous variables are expressed as mean±SD, and categorical variables are expressed as percentages. The $\chi 2$ test will be used to see the relationship between dichotomous variables, and Student's t-test will be used for continuous variables. Single-variable correlation analysis and multivariable linear regression analysis will be performed. A P value <0.05 is considered statistically significant. The analyses will be performed with SPSS for Windows.

Ethics and Dissemination

The ethics of this study were approved by the ethics committee of National Cardiovascular Center Harapan Kita (NCCHK), Jakarta, Indonesia, with ethical code LB.02.01/VII/286/KEP.009/2018. This study has been registered at clinicaltrials.gov with the identifier code NCT03991910.

DISCUSSION

This study is planning to recruit rheumatic mitral valve patients to be randomised to obtain capsules containing either ramipril 5 mg or placebo. Rheumatic mitral stenosis is the main presentation of RHD that leads to significant morbidity and mortality. Recurrent or persistent valvulitis with bicommisural fusion usually leads to mitral stenosis. Previous studies suggest that RHD is an autoimmune disease that is associated with cytokine activities. Inflammatory cytokines are key regulators of immune processes.[4] Immunologic reactions caused by autoreactive antibodies continuously cause chronic inflammation and valvular fibrosis, which can be detected by an increase in sST2, an emerging biomarker for cardiac fibrosis.[10,15,16]

IL-33 is the natural ligand of ST2 and is highly expressed in smooth muscles and airway epithelia.[17] An inflammatory state stimulates the upregulation of ST2 by some cells, such as keratinocytes and dermal fibroblasts, and mechanical strain upregulates ST2 in cardiac fibroblasts.[17,18] The soluble ST2 isoform is increased under inflammatory conditions such as sepsis, allergic asthma, trauma, and pulmonary diseases.[19–22] Its elevation is also documented in some heart conditions, such as aortic stenosis and congestive cardiomyopathy, and this elevation is associated with the risk of heart failure and death.[23–27] In this study, plasma ST2 is considered an inflammatory and fibrotic biomarker of rheumatic mitral stenosis. Because plasma ST2 can also increase in various conditions unrelated to cardiac fibrosis, this study also measures the ST2 expression in mitral valve tissue. Plasma ST2 describes the

amount of ST2 in the circulation, whereas mitral valve cells that express ST2 describe the amount of transmembrane ST2.

ACEIs are commonly administered as the treatment of heart failure due to valvular regurgitation. Its use in MS is still debatable because of its hypotensive effect. A prior study assessing the safety of ACEIs in MS patients showed that the ACEI enalapril was well tolerated and safe up to a dose of 10 mg bid.[11] ACEIs are presumed to have vasodilatory effects in obstructive lesions and will decrease systemic vascular resistance through arterial vasodilatation, thus increasing the transvalvular gradient. Their anti-remodelling effect is also well established, and their long-term use has also been proven to improve left ventricular ejection fraction (LVEF) in patients with systolic dysfunction.[28] Because a prior study[11] demonstrated the efficacy and the potential benefits of ACEIs in improving outcomes in MS patients, this study aims to confirm and investigate the possible pathological mechanism of those improvements. This study will assess the effect of 5 mg ramipril as a cardiac antifibrosis treatment in severe MS RHD patients. Their plasma ST2 concentrations will be compared. Plasma ST2 concentration will also be compared before and after several months of consuming 5 mg ramipril. There will be no healthy controls for this study because of ethical limitations in the acquisition of mitral valve tissue. Mitral valve tissue will be acquired during mitral valve surgery. The expression of ST2 in mitral valve tissue will then be calculated semi-quantitatively and compared with the plasma ST2 results. It is hypothesised that ramipril will suppress the expression of ST2 in the cardiac mitral valve in patients with RHD.

In addition to the plasma ST2 level and the ST2 expression in mitral valve tissue, this study also compares the pre-post effects of 5 mg ramipril versus placebo on NT-proBNP concentration echocardiography strain parameters and clinical outcomes. Clinical signs and symptoms and echocardiography parameters have been evaluated in some studies of mitral valve stenosis, and showed that these were positively correlated with the NT-proBNP concentration.[29,30] This study will also compare the NT-proBNP concentration between patients receiving ramipril and placebo. We will also calculate the correlation between the NT-proBNP concentration and the ST2 plasma concentration and mitral valve expression.

Figure legends

Figure 1 Hypothesis.

Molecular mimicry is a defense mechanism of group A streptococcus to avoid immune cells. This mechanism allows immune cells to generate autoimmunity against protein the lining of endothelial cells and causing chronic inflammation and valvular damage. Continous proccess of chronic inflammation leads to valvular thickening and fibrosis, which is mediated by the Angiotensin II. Angiotensin II increase TGF-β expression and cause IL-33 to bind with sST2, and subsequently cause damage and fibrosis to the valvular tissue evenmore, which later will ended with rheumatic heart failure. ACEI is hypothized to counteract these processes by decreasing Angiotensin II conversion from Angiotensin I.

Figure 2 Research Flowchart

Authors contributions

Conception and design of the work was initiated by AMA, BS, AS, BR, and BD. AMA, BD, ES, and FT contributed to the acquisition, analysis, and interpretation of data for the work. This manuscript was drafted by AMA, BD, and ES. AMA, PD, AW, and MJC critically revised the manuscript. The author and coauthors gave final approval and agree to be accountable for all aspects of the work and ensuring its integrity and accuracy.

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Competing interests statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Patient and Public Involvement statement

Patients are not involved in the recruitment to and conduct of this study protocol.

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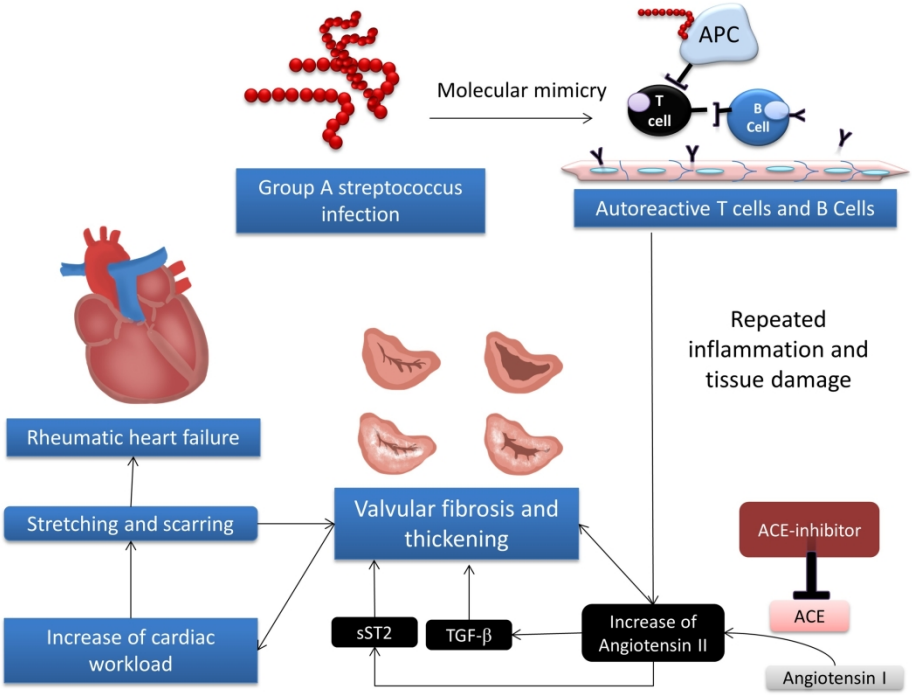

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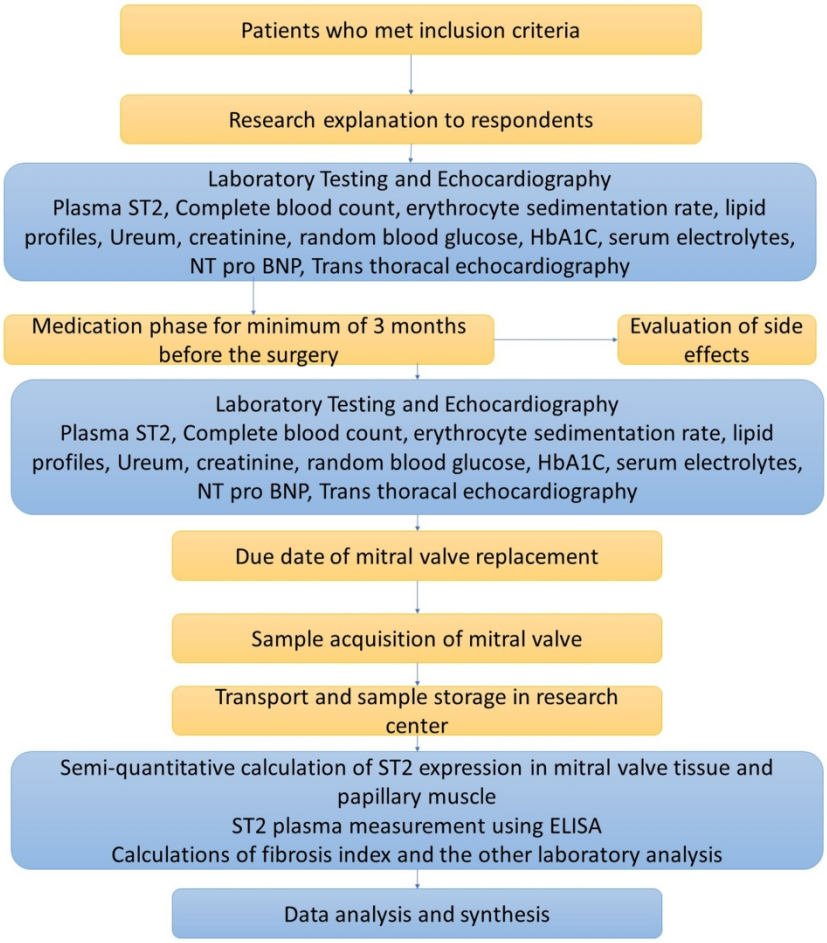

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Randomised Controlled Trial into the role of ramipril in fibrosis reduction in RHD: The RamiRHeD trial protocol
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Clinicaltrials.gov NCT03991910

2b All items from the World Health Organization Trial Registration Data Set

Register	ClinicalTrials.gov
Main ID	NCT03991910
Protocol ID	RamiRHeD
Date of Registration	19/06/2019
Prospective Registration	Yes
Primary sponsor	Harapan Kita National Cardiovascular Center/Indonesia University
Public title	The Effect of Ramipril in Suppressing ST2 Expression in Rheumatic Mitral Stenosis Patients
Scientific title	The Effect of Ramipril in Suppressing Gene Expression of Fibrosis in Cardiac Mitral Stenosis in Patients With Rheumatic Heart Disease
Date of first enrloment	June 27, 2019
Target sample size	66
Recruitment status	Recruiting
Study type	Randomised clinical trial
Study design	Allocation: Randomized. Intervention model: Parallel Assignment. Primary purpose: Treatment. Masking: Double (Participant, Investigator).
Phase	Phase 3
Countries of Recruitment	Indonesia
Health condition	ACE inhibitor Fibrosis; heart Mitral stenosis Rheumatic heart disease Rheumatic mitral stenosis
Intervention (s)	Drug: placebos Drug: Ramipril 5 mg oral capsule
Primary outcome	ST2 expression in mitral valve tissue
Secondary outcome	ST2 plasma level NT-proBNP concentration (pg/ml) Ejection fraction TAPSE (tricuspid annular plane systolic excursion NYHA class

Protocol version 3 Date and version identifier

Released: 19-06-2019

Funding 4 Sources and types of financial, material, and other support

No specific fundings outside our institution (Harapan Kita National Cardiovascular Center, Indonesia)

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Ade M. Ambari • Budhi Setianto• Anwar Santoso• Basuni Radi • Bambang Dwiputra Department of Cardiology and Vascular Medicine Faculty of Medicine University of Indonesia, National Cardiovascular Center Harapan Kita, Jakarta, Indonesia Eliana Susilowati •Fadilla Tulrahmi Research Assistant Division of Preventive and Rehabilitative Cardiology, National Cardiovascular Centre Harapan Kita, Jakarta, Indonesia Maarten .J.M. Cramer • P.A. Doevendans • Annemiek Wind Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands Role: Ade M. Ambari conceived the study. Ade M. Ambari, Pieter A. Doevendans, Maarten .J.M. Cramer, Budhi Setianto, Anwar Santoso, Basuni Radi, Bambang Dwiputra initiated the study design and conceptual framework. Ade M. Ambari, Bambang Dwiputra contribute in patients' recruitment and assessment,writing and editing. Eliana Susilowati contribute to the biomedical methods, sample collection, writing and editing. Fadilla Tulrahmi conducting the primary statistical analysis, randomisation, and sample size calculation. Ade M. Ambari, Pieter A. Doevendans, Maarten .J.M. Cramer, Annemiek Wind contribute to writing, protocol editing, language editing. All authors contributed to refinement of the study protocol and approved the final manuscript.
	5b	Name and contact information for the trial sponsor Trial sponsor: Harapan Kita National Cardiovascular Center, Jakarta Contact name: Ade Meidian Ambari Address: S Parman Street number 87, Special Region of Jakarta, Indonesia Telephone: 0215681111 Email: dr_ade_meidian@yahoo.co.id
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

Introduction

Principal Investigators
Design and conduct of RCTRHDMS
Preparation of protocol and revisions
Organising steering committee meetings
Publication of study reports
Members of TMC [*Trial Management Committee*]

Steering committee (SC)
Agreement of final protocol
All lead investigators will be steering committee members
Reviewing progress of study and if necessary agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study.

Trial Management Committee (TMC)
(Principle [*sic*] investigator, Research Physician, Administrator)
Study planning
Organisation of steering committee meetings
Provide annual risk report to ethics committee
report serious adverse events (SAE) to medical committee and ethics committee
Responsible for trial master file
Budget administration and contractual issues with individual centres
Advice for lead investigators
Audit of 6 monthly feedback forms and decide when site visit to occur.
Assistance with international review, board/independent ethics committee applications
Data verification
Randomisation
Organisation of central serum sample collection

Data Manager
Maintenance of trial IT system and data entry
Data verification

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Rheumatic heart disease (RHD) is a major burden in developing countries home to 80% of all people living with the disease where it causes most of the cardiovascular morbidity and mortality in children and young adults. Chronic inflammation and fibrosis of heart valve tissue due to chronic inflammation in RHD will cause calcification and thickening of the impacted heart valves, especially the mitral valve. This fibrogenesis is enhanced by the production of Angiotensin II by increasing TGF-β expression and latter, the binding of IL-33 which is known to have anti-hyperthropic and anti-fibrotic effects to sST2. Its binding to the non-natural ligand of sST2 will worsen the fibrosis. Therefore, we hypothesized that Angiotensin-converting enzyme inhibitor (ACEI) will improve rheumatic mitral valve stenosis.

Existing knowledge: Angiotensin-converting enzyme inhibitors (ACEI) are first-line therapy in cardiac failure, and their symptomatic and survival benefits extend beyond afterload reduction. Reduction in fibrosis and anti-proliferative and neurohumoral effects contribute to the ACEI effect that is not reproduced by pure vasodilators. ACEI was well tolerated in symptomatic RHD associated with significant mitral stenosis and preserved left the ventricular systolic function. New therapies targeting ST 2 and their receptors as studied in some autoimmune diseases may promise a new approach for patients with RHD. We are assessing the effect of Ramipril in suppressing fibrotic protein expression in mitral valve (measured with ST2 expression) of patients with RHD in the National Cardiac Center Harapan Kita hospital Jakarta Indonesia.

Dose selection: Prevoious study (SCOPE trial) showed that ACEI (Enalapril) was well tolerated in symptomatic RHD associated with significant aortic and/or mitral stenosis and preserved left ventricular systolic function until dose of 10 mg bid. This study use Ramipril 5 mg as it is more commonly used in Indonesia.

Need for a trial: Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. In developing countries, percutaneous balloon mitral valvuloplasty and mitral valve surgery are the therapeutic options for rheumatic mitral stenosis. Both of these treatments involve enormous expenses; it is the public health cost burden for developing countries. Valvular anti-inflammatory and anti-fibrosis medical therapy to suppress the progression of the disease is needed in rheumatic mitral stenosis patients.

	6b	Explanation for choice of comparators Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. This study is divided in 2 arms. The first arm will be given Ramipril 5 mg as the intervention, while the second arm, will be given placebo as the comparator. The other individualized treatments for rheumatic valvular disease was still given in both armsas indicated.
Objectives	7	Specific objectives or hypotheses The investigators hypotized that administration of Ramipril 5 mg for 3 months will reduce expression of ST2 as fibrosis biomarkers, in the cardiac mitral valve of patients with Rheumatic Heart Disease with mitral stenosis.
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) This is a single-centre, double-blind, placebo-controlled, pre-post test design, randomised clinical trial. Patients with mitral stenosis valvular dysfunction due to rheumatic process planned for cardiac valve replacement were given Ramipril 5 mg or placebo for minimum 12 weeks before the surgery. ST2 was checked as fibrosis marker. This study will be conducted in the Department of Cardiology and Vascular Medicine, University Indonesia, National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia from June, 27th 2019.

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Study setting: in a national academic hospital. Patients come from various regions in one country (Indonesia).
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) • Inclusion criteria of this study are: Patients with mitral valve stenosis or a combination • aged more than 18 years • undergo cardiac valve replacement operation with or without a tricuspid valve repair, • patients with systolic blood pressure (SBP) ≥ 100 mmHg and diastolic blood pressure (DBP) ≥ 60 mmHg • passed in medication phase without side effect minimum 4 weeks until operation schedule

Interventions

11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered

Arms and Interventions

Arms	Interventions
Placebo Comparator: control control patients will be given a placebo	Drug: Placebos the control group will be given placebo inside a capsule, so study participant won't be able to know the drug and doses inside the capsule (for masking)
Experimental: treatment Ramipril 5 mg treatment group	Drug: Ramipril 5Mg Oral Capsule the treatment group will be given Ramipril 5 mg inside a capsule, so study participant won't be able to know the drug and doses inside the capsule (for masking)

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)

Participants will be informed that they are able to withdraw from the study at any time and will sign a reference as proof. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry. Drop out criteria will be Participants who are lost to follow up, participants with severe adverse events, mortality due to any cause.

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)

Patients' adherence will be monitored with the drug return. Patients will be contacted via phone number to be reminded for routine administration. Patients are provided with the investigator contact if needed something to ask or to report adverse events or side effects (if any). Patients will be asked to visit the hospital where this study is enrolled to meet the investigator monthly. Each month, capsule return will be counted and patient will also be evaluated for the symptoms and vital signs.

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial

Rescue medication: stop the drug. Patient will be admitted to hospital and undergone treatments according to the adverse effects.

Prohibited concomitant medication: Angiotensin receptor blocker.

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended

Primary Outcome Measures :

- ST2 plasma level

plasma level of ST2 measured by ELISA

Secondary Outcome Measures :

- ST2 expression in mitral valve tissue

expression of ST2 in mitral valve tissue, using immunohistochemistry method

- NT-proBNP concentration (pg/ml)

concentration of NT-proBNP, plasma markers for cardiac dysfunction.

- ejection fraction

echocardiography parameter

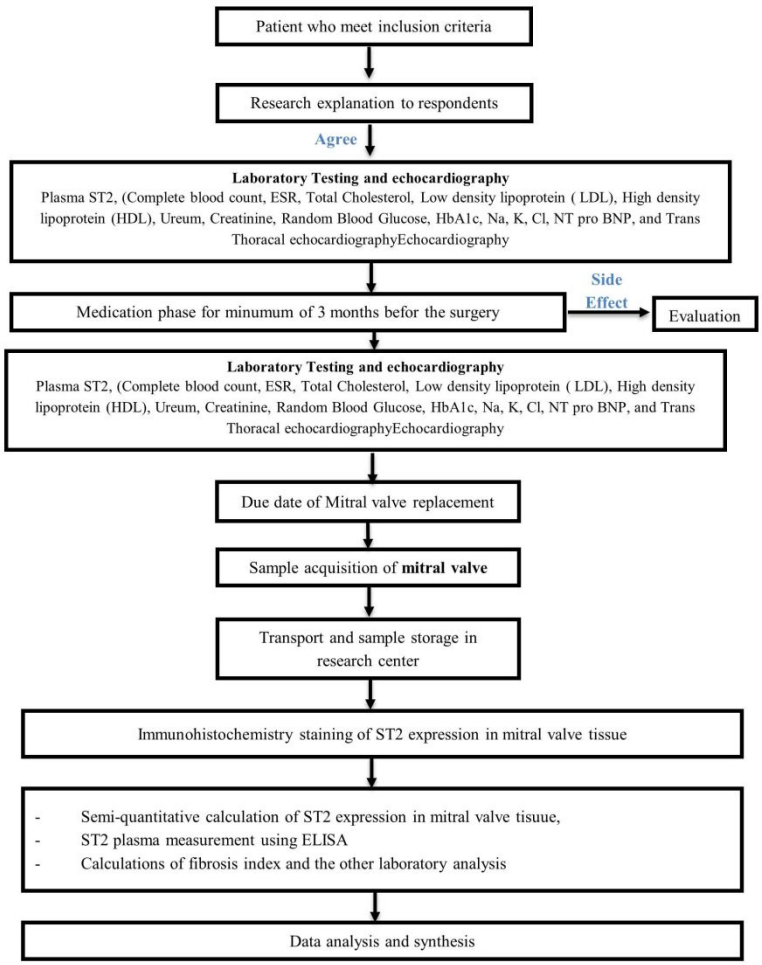
- TAPSE (tricuspid annular plane systolic excursion)

echocardiography parameter to asses right ventricular function

- NYHA class

related symptoms will be graded in class I to IV according to NYHA.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)



Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations This is a pioneer study for analysing effects of Ramipril 5 mg toward ST2 expresion in mitral valve tissue in human. Previous study that use ST2 human tissue is a study from Marzullo et at in 2016[12] that use carotid tissue from carotid endarterectomy, with sample size of 41 consecutive patients. Because our study will use human tissue sample, we approached the sample size calculation using multistage non-finite population method, using this specified precision estimation formula[13]: $N = (Z\delta)/E$, with N= sample size; $Z_{0.95}$= 1.96; δ N(0,1) = 1; and E= 0.05 for 0.95 confidence interval. So we calculate the sample and found that 1.65(1)/0.05= 33 samples. According to the sample size of the previous study that analyse ST in human tissue and a sample size formula that commonly used in in-vivo study, we decide to use a sample size of 30 for each arm, and with the addition of drop out rate of 10%, become total of 66 for 2 arms. The number includes a 10% dropout and withdrawal in each group. Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Patient recruitment will involves several cardiologists and cardiothoracic surgeons. All cardiologists and cardiothiracic surgeons in the hospital will be informed about this study, and to inform back if their patient suffered from Mitral valve stenosis. Technical meetings will be held and the profesionals related to this study will be invited.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Allocation sequence generation are from online, web-based sequence generator system. Participants will be enrolled with staff member responsible for patients enrollment.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Trial participants, investigators, analysts, care providers, will be blinded. Reserach assisstants whose role are to follow-up, evaluate and monitor the patients condition will not be blinded, so the drug could be stopped easily if there is any adverse effect from the treatment group.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Under the circumstances where actual treatment is absolutely necessary for further management of the patient, unblinding is permissible.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Data collections will involve profesional in each filed of data collection. For example: patients will be diagnosed with experienced cardiologists, surgery desicion based on the decision of a multidisciplinary team, echocardiography will be performed by the cardiologist who is specialized in echocardiography. Blood samples will be drawn by the experienced nurses in pathology clinic laboratory, ST2 plasma and tissue will be collected with the biomedical anlalysts, interview of study participants will be done by the trained assessors which. Study instruments involve questionnaires, laboratory tests, echocardiography, and biochemical tests. Study instruments will use same technique, same tools, same brands, and same place for data collection of each study participant.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Participants will be informed that they are able to withdraw from the study at any time and will sign a reference as proof. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Participant files will be stored in numerical order and stored in a secure and accessible place and manner. Participants data will be copied in softfile and have back-up data.

Statistical methods 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol

Variable/Outcome	Hypothesis	Outcome Measure	Methods of Analysis
1) <u>Primary</u>	Participants adherence of Ramipril 5 mg for 3-6 months or placebo 3-6 months will be 80%	Remaining drug in the participants is not more than 20% of the study drug given, to evaluate the adherence.	Manual counting, univariate analysis
a) ST2 expression in mitral valve tissue	Reduction occurr	Mitral valve tissue ST2 measured using percentage of-cell-expressing ST2 using immunohistochemistry method	T-test
2) <u>Secondary</u>			
a) ST2 plasma level	Reduction occurr	Plasma ST2 concentration will be measured with the concentration calculated according to the absorbance in ELISA technique	T-test
b) NT-proBNP concentration	Reduction occur	NT-proBNP concentration is measured in clinical pathology laboratorium using ELISA.	T-test
c) Ejection fraction(EF) and TAPSE	Improvement occurr	Measure was performed using same echocargiography pre and post test, documented in percentage.	T-test
d) NYHA class	Reduction occurr	NYHA class will be measured accoding to the clinical signs and symptoms classified in NYHA	Chi-square test

20b Methods for any additional analyses (eg, subgroup and adjusted analyses)

Continuous variables were expressed as mean±SD and categorical variables as percentages. The $\chi 2$ test was used to see the relationship between dichotomous variables and the Student t-test for continuous variables. Single variable correlation analysis and multivariable linear regression analysis will be performed. A P value <0.05 was considered statistically significant. The analyses were performed with the use of SPSS for Windows.

20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

This study will be analysed in per-protocol fashion. Handling missing data will use multiple imputation method. Analysis of the primary endpoint will be based on a log-rank test and, therefore, not affected by patient withdrawals (as they will be censored) provided that dropping out is unrelated to prognosis. The effect that any missing data might have on results will be assessed via sensitivity analysis of augmented data sets. Dropouts (essentially, participants who withdraw consent for continued follow-up) will be included in the analysis by modern imputation methods for missing data. After the imputations are completed, all of the data (complete and imputed) will be combined and the analysis performed for each imputed-and-completed dataset. Rubin's method of multiple imputation will be used to estimate treatment effect.

Methods: Monitoring

Data monitoring

21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

A Data Monitoring Committee (DMC) has been established. The DMC is independent of the study sponsor. During the period of recruitment to the study, interim analyses will be supplied, in strict confidence, to the DMC, together with any other analyses that the committee may request.

21b Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial

An interim-analysis is performed on the primary endpoint when 80% of patients have been randomised and mitral valves have been obtained. The interim-analysis is performed by a statistician in this study member. Stopping decision (if needed) based on the desicion of the primary investigators and will be reported to the ethics commitee.

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Adverse events that happen before the patient started to receive study intervention will not be reported as it is not related to the study drug. All adverse events occurring after the patient receiving study intervention until the end of the study will be recorded. Serious adverse event (SAE) related to this study treatment will be reported to the institutional review board and ethical committee. Serious adverse event including: life-threatening condition with immediate risk of death, severe or permanent disability, prolonged hospitalisation, or a sgnificant hazard determined by the data safety monitoring board. SAE that is believed by the investigator and medical committee to be causally related to the study drug will be reported. SAE occuring a month after the subject is discontinued from the study will not be reported unless the investigators believed that the event have been caused by the study drug. The causal effects will be determined according to the temporal relationship, clinical course, previous medical conditions and concomitant medications.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor Ethic committee will audit the trial conduct

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval This protocol and the template informed consent forms contained in Appendix I has been reviewed and approved by the IRBs/ECs [*institutional review boards/ethical committees*] with respect to scientific content and compliance with applicable research and human subjects regulations. The protocol, site-specific informed consent forms is in local language. Participant education and recruitment materials, and other requested documents and any subsequent modifications also will be reviewed and approved by the ethical review bodies (IRBs/ECs).

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. If such amandement happens, it will request an approval of the Ethics Committee/IRB [*institutional review board*] prior to implementation.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) The member of investigators will explain and obtain informed consent.
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Additional biological samples will be obtained to be stored for use in future studies. It will be stored in research center inside this hospital with adequate and certified samples handling. A materials consent will be obtained to the collection of the plasma speciments.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial All study-related information will be stored securely in the principal investigator's locker with limited access. All participants data will be identified by a coded ID number to maintain the confidentiality. Laboratory speciments will be stored in research center with a safe storage and will be identified also with coded ID. Local database will be secured with password access systems and the access will be limited.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site All of the investigators disclose no conflict of interests.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Data management coordinating team will oversee the intra-study data sharing process. All principal investigators will be given access to the cleaned datasets. Principal investigators will have direct access to their own data sets and will have access to other sites data by request. To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Study participants are covered by compensation for negligent harm through the standard of this hospital. This will include cover for additional health care, if it has causal relationship with the study drug.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Investigators is not expected to report the data as individual report. All presentations and publications are expected to protect the integrity of the major objectives of the study; data that break the blind will not be presented prior to the release of the main results. Recommendation as to the timing of presentation of endpoint data which they might be presented will be given by the steering committee. Each paper abstract must be submitted to the appropriate subcommittee for review of its appropriateness and scientific merit prior to submission, the subcommittee may recomend changes to the authors and submit its recommendations to be approved by the steering committee. Publications of papers to workshops, symposia, volumes etc will be in the right of the principal investigators, and principal investigators could appoint and give permission for the other investigators or other party to present this paper. the study results will be released to the participating physicians, patients, and general medical community.
	31b	Authorship eligibility guidelines and any intended use of professional writers Topics suggested for presentation or publication will be circulated to the PIs [*Principal investigators*] of the CCCs [*Core Coordinating Centers*], the DCC [*Data Coordinating Center*], and research center in hospital. These groups are requested to suggest and justify names for authors to be reviewed by the PC [*Publications Committee*].
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code Data sharing statement: no later than 5 years after the collection of the 1-year post randomisation interviews, we will deliver a completely deidentified data set to an appropriate data archive for sharing purposes.

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates

RAHASIA 1

Lembar Informasi dan Persetujuan Pasien

Judul Penelitian :

The Effect of Ramipril in Suppressing Gene Expression of Fibrosis in Left Atrial Appendage in Cardiac Mitral Stenosis Rheumatic Heart Disease Patients

Latar Belakang dan Tujuan Penelitian

Penyakit jantung rematik merupakan beban penyakit utama di negara berkembang yang hampir 80% seluruh orang hidup dengan penyakit ini, dimana sebagai penyebab tingginya angka kematian dan kesakitan kardiovaskular pada anak dan remaja. Berdasarkan *Global Burden of Disease Study* (2010), jumlah pasien yang mengalami disabilitas karena penyakit jantung rematik sebanyak 10,1 juta per tahun di seluruh dunia.[1] *Rheumatic Mitral Valve Stenosis* (RMS) merupakan indikator utama penyakit jantung rematik yang dapat berdampak signifikan pada kematian dan kesakitan.

ST2 merupakan bagian reseptor IL-1 yang terdiri dari 2 bentuk, *a trans-membrane receptor* (ST2L) dan *soluble decoy receptor* (sST2).[4] Keduanya diinduksi di *cardiomyocyes* dan *fibroblast* yang terpapar tekanan biomekanik. Fungsi dari ST2 pada penyakit kardiovaskular, IL-33 telah terbukti memiliki efek anti-hipertrofik dan anti-fibrotik pada jantung, ditransduksi oleh ST2L.[4]

Angiotensin-Converting Enzyme Inhibitors (ACEI) sering digunakan untuk mencegah dan mengobati gagal jantung karena penyakit katup regurgitasi. Mayoritas pasien dengan penyakit jantung rematik simtomatik (RHD) memiliki mitral stenosis (MS) yang signifikan dan menolak terapi ACEI, hal ini disebabkan karena ditakutkan adanya hipotensi di kemudian hari dengan adanya obstruksi tetap.[5] ACEI pula dapat ditoleransi pada penyakit jantung rematik simtomatik berkaitan dengan mitral stenosis yang signifikan dan tetap mempertahankan fungsi sistolik ventrikel kiri.[5]

Efikasi pencegahan sekunder terbatas dalam mencegah progresivitas penyakit jantung rematik sehingga diperlukan adanya strategi dan terapi yang dibutuhkan untuk mencegah hal tersebut.[3] Terapi terbaru menargetkan ST2 dan reseptor seperti yang diteliti pada penyakit autoimun memungkingkan adanya pendekatan baru untuk pasien penyakit jantung rematik. ACEI merupakan agen dengan efek anti fibrosis. Oleh karena itu, peneliti ingin mengetahui efek Ramipril dalam memodulasi ekpresi gen fibrosis pada

jaringan katup dan appendiks atrium kiri pasien dengan penyakit jantung rematik di RS Jantung dan Pembuluh Darah Harapan Kita.

Penjelasan tentang Prosedur Pelaksanaan

Pasien dengan penyakit jantung rematik mitral stenosis yang akan dilakukan tindakan MVR/r akan diberikan Ramipril atau Placebo selama minimal 3 bulan dan maksimal 6 bulan yang akan dikonsumsi setiap hari sampai waktu untuk dilakukannya tindakan operasi.

Partisipasi sukarela/pengunduran diri

Partisipasi anda dalam penelitian ini bersifat sukarela, anda dapat menolak untuk berpartisipasi. Jika anda memutuskan untuk berpartisipasi, maka anda akan diberi lembar informasi ini untuk dipelajari isi dan tujuan penelitian. Anda dapat menyimpan lembar informasi ini dan anda akan diminta untuk mengisi dan menandatangani formulir persetujuan ini.

Anda dapat mengundurkan diri di awal saat anda membaca informasi dari penelitian ini dan tidak menyetujui untuk mengikuti penelitian tanpa harus memberikan alasan. Pengunduran diri anda tidak menimbulkan sanksi apapun dan anda tidak akan kehilangan manfaat yang akan menjadi hak anda.

Manfaat partisipasi

Secara pribadi anda dapat mengambil manfaat berpartisipasi dalam penelitian ini karena dapat membantu mengurangi gejala dan tanda penyakit jantung katup mitral rematik. Anda juga dapat memberikan manfaat bagi orang lain dalam pengembangan ilmu pengetahuan dan peningkatan kesehatan masyarakat secara luas.

Resiko dan Ketidaknyamanan

Konsekuensi dari partisipasi ini mengharuskan anda untuk minum obat maksimal selama 6 bulan setiap hari sebelum dilakukannya tindakan MVR/r. Partisipan akan melakukan pengambilan obat setiap bulannya ke tempat penelitian. Selain itu juga akan menjalani beberapa pemeriksaan penunjang medis seperti echocardiography dan

pengambilan darah yang akan dilakukan sebelum fase minum obat, dan setelah selesai minum obat.

Kerahasiaan

Semua data pada penelitian ini akan diambil tanpa memberikan identitas anda. Kerahasiaan data dan identitas Anda dilindungi oleh hukum dan atau peraturan yang berlaku, dan tidak akan diberitakan secara umum. Pada saat hasil diumumkan, identitas Anda akan tetap terjaga kerahasiaannya.

Hanya pihak yang terlibat dalam penelitian ini saja yang akan diberikan wewenang untuk dapat memperoleh dan mengetahui keadaan kesehatan Anda, termasuk didalamnya dokter Anda dan perawat, rumah sakit, pihak sponsor dan perwakilannya, dan atau anggota dari Komisi Etik. Anda mempunyai hak untuk mendapatkan segala informasi yang berhubungan dengan keikutsertaan Anda dalam penelitian ini.

Persetujuan Komite Etik Kedokteran

Penelitian ini diteliti dan disetujui oleh Komisi Etik Pusat Jantung Nasional Harapan Kita.

Biaya

Partisipan yang mengikuti penelitian ini akan diberikan biaya perjalanan/transportasi dari rumah ke tempat penelitian sebesar Rp. 100.000,- pada setiap bulan selama masa konsumsi obat. Biaya penelitian ditanggung oleh peneliti yang termasuk dalam paket penelitian.

Lain-lain

Jika anda merasa tidak nyaman, anda dapat memilih untuk tidak ikut serta dalam penelitian. Hal ini tidak akan mempengaruhi pelayanan rumah sakit terhadap anda di masa mendatang

Penawaran untuk Menjawab Pertanyaan

Jika Anda mempunyai pertanyaan-pertanyaan mengenai studi ini, Anda dapat menghubungi:

dr. Ade Meidian Ambari, SpJP(K)
No. telepon. 021 – 568 4085 ext 2209

Silahkan untuk tidak mendatangani formulir ini jika anda tdak mempunyai kesempatan untuk bertanya atau tidak menerima jawaban-jawaban yang memuaskan terhadap pertanyaan-pertanyaan anda.

Pernyataan Persetujuan

Dengan menandatangani formulir ini, saya menyetujui bahwa penelitian ini telah dijelaskan kepada saya dan semua pertanyaan saya telah dijawab dengan memuaskan. Saya juga mempunyai hak untuk dapat mengundurkan diri dari penelitian ini setiap saat. Dengan pengertian tersebut, saya dengan sukarela ikut serta dalam penelitian ini. Saya mengerti bahwa formulir ini akan disimpan bersama dengan data kesehatan saya dan saya akan mendapatkan *copy* dari formulir ini.

Nama Pasien : _____	Nama Wali : _____
Tanda tangan : _____	Tanda tangan : _____
Tanggal : ___/___/_____	Tanggal : ___/___/_____

Nama Dokter / Asisten : _____
Tanda tangan : _____
Tanggal : ___/___/_____

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Plasma speciments will be stored in biobank inside the research center in this hospital, and has been approved by the medical and ethical committee for the possible further researches.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Randomised Controlled Trial into the role of ramipril in fibrosis reduction in Rheumatic Heart Disease: The RamiRHeD trial protocol

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Randomised Controlled Trial into the role of ramipril in fibrosis reduction in Rheumatic Heart Disease: The RamiRHeD trial protocol

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ABSTRACT

Introduction Rheumatic heart disease (RHD) is a major burden in developing countries and accounts for 80% of all people living with the disease, where it causes most cardiovascular morbidity and mortality in children and young adults. Chronic inflammation and fibrosis of heart valve tissue due to chronic inflammation in RHD will cause calcification and thickening of the impacted heart valves, especially the mitral valve. This fibrogenesis is enhanced by the production of angiotensin II by increased TGF-β expression and later by the binding of IL-33, which is known to have anti-hypertrophic and anti-fibrotic effects, to soluble sST2. sST2 binding to this non-natural ligand worsens fibrosis. Therefore, we hypothesise that angiotensin-converting enzyme inhibitors (ACEIs) would improve rheumatic mitral valve stenosis.

Methods and analysis This is a single-centre, double-blind, placebo-controlled, randomised clinical trial with a pre-post test design. Patients with rheumatic mitral stenosis and valve dysfunction will be planned for cardiac valve replacement operation will be given ramipril 5 mg or placebo for a minimum of 12 weeks before the surgery. The expression of ST2 in the mitral valve is considered to be representative of cardiac fibrosis. Mitral valve tissue will be stained by immunohistochemistry to ST2. Plasma ST2 will be measured by ELISA. This study is conducted in the Department of Cardiology and Vascular Medicine, Universitas Indonesia, National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia, starting on June 27th, 2019. The performance and dissemination of this study were approved by the ethics committee of National Cardiovascular Center Harapan Kita with ethical code LB.02.01/VII/286/KEP.009/2018. This study has been registered at clinicaltrials.gov with the identifier code NCT03991910.

Strengths and limitations of the study

- A novel study that analysed the ST2 expression in rheumatic heart patients' mitral valves.
- This study proposed novel and affordable treatment targeting the rheumatic heart valve fibrosis reduction.
- This research will help low-to-middle-income countries treat rheumatic heart disease more economically.
- Flexible schedule of mitral valve surgery causes different time range of the intervention for each patient.
- No standard healthy control of the non-fibrotic valve, based on ethical consideration.

INTRODUCTION

Rheumatic heart disease (RHD) is a serious health problem in developing nations, where it affects 80% of the population and accounts for the majority of cardiovascular morbidity and mortality in children and young adults. RHD affects more than 15.6 million individuals worldwide, with 233,000 people dying prematurely each year. [1] In the past 5 years, approximately 471 rheumatic mitral stenosis patients were treated in our Centre. [2] Treatments provided for RHD in advanced stages are relatively expensive for developing nations; thus, early detection and targeted treatment can greatly aid.[3] Mitral valve stenosis is the main presentation of RHD, commonly developing as a result of persistent or recurrent valvulitis with bicommissural fusion.[4] Fibrogenesis is induced by various stimuli, such as cytokines, connective tissue growth factors, and activators. Previous studies suggest that RHD is an autoimmune disease that is associated with cytokine activation.[4] Inflammatory cytokines are key regulators of immune processes. Chronic inflammation causes damage to the valvular tissue. Many studies have investigated potential biomarkers to evaluate fibrosis and chronic inflammation processes in RHD patients, and ST2 is a sensitive marker for detecting cardiac fibrosis, including fibrosis progression in RHD.[4–6]

ST2 is a member of the interleukin (IL)-1 receptor family discovered in a classical translational science fashion, and it exists in two forms: a transmembrane receptor (ST2L) and a soluble decoy receptor (sST2).[7] As a member of the interleukin (IL)-1 receptor family, ST2 is a biomarker of mechanical stress that is up-regulated in isolated cardiomyocytes exposed to mechanical strain; derangement of ST2 signalling leads to a phenotype consistent with myocardial remodelling, and in patients with heart failure, sST2 levels strongly correlate with the severity of heart failure, independently forecasting risk on top of the risk from NT-proBNP and other biomarkers. Both sST2 and ST2L are induced in cardiomyocytes and fibroblasts exposed to biomechanical stress. Biomechanical stress and fibrosis will enhance valve thickening in RHD.[8] Clarifying the role played by ST2 in cardiovascular disease, IL-33 signalling through ST2L has been shown to have anti-hypertrophic and anti-fibrotic effects in the heart.[7] Calcification and thickening of the mitral valves are enhanced by the production of angiotensin II. Angiotensin II induces the upregulation of transforming growth factor β (TGF-β) and later the binding of IL-33 to sST2 instead of to its natural receptor ST2L. Binding of IL-33 to sST2 will cause fibrogenesis. Thus, ACEIs are hypothesised to attenuate this vicious cycle by inhibiting angiotensin II and consequently increasing bradykinin, which further inhibit fibrosis through the negative regulation of angiotensin II activity in mitogen-activated protein kinase (MAPK) pathways through the suppression of the Ca2+ response and Na+ transport.[9,10]

ACE inhibitors are frequently used to prevent and treat heart failure caused by regurgitant valve disease. Because of the risk of hypotension in the presence of a fixed obstruction, the majority of patients with symptomatic RHD have substantial mitral stenosis (MS) and refuse ACEI medication.[11] ACEI is the primary treatment for heart failure. The way ACEIs improve clinical symptoms and survival outcomes is to advance afterload reduction. Fibrosis attenuation and its anti-proliferative effects and neurohormonal effects are superior to those of pure vasodilators.[11] Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. The only established therapeutic options for rheumatic mitral stenosis are balloon mitral valvuloplasty and mitral valve surgery. More economical therapeutic options that target the inhibition of fibrogenesis and improve mitral valve fibrosis are needed, especially in low- to middle-income countries. Valvular anti-inflammatory and anti-fibrotic medical therapy to slow the progression of the disease is needed in rheumatic mitral stenosis patients. One ACEI (enalapril) was well tolerated in symptomatic RHD associated with significant aortic and/or mitral stenosis and preserved left ventricular systolic function.[11]

Currently, there is no treatment for rheumatic mitral stenosis that targets the main pathogenesis, valvular fibrosis. Therefore, novel approaches and therapies are needed to prevent RHD progression.[4] Neutralising inflammatory cytokines or antagonising their receptor function has been considered a useful therapeutic strategy to treat autoimmune diseases.[4] In this respect, new therapies targeting ST2 and its ligands, as studied in some autoimmune diseases, may be a new approach for patients with RHD. ACEIs are agents with anti-fibrotic effects. This study therefore aims to investigate the effect of the ACE inhibitor ramipril in suppressing the expression of ST2 in the cardiac mitral valve in patients with RHD (Figure 1).

METHODS AND ANALYSIS

Study Designs

This is a single-centre, double-blind, placebo-controlled, randomised clinical trial with a pre-post test design. Rheumatic mitral stenosis patients with valvular dysfunction who are scheduled for cardiac valve replacement will be treated with ramipril 5 mg or placebo for a minimum of 12 weeks (3 months) before the surgery. ST2 will be checked as a fibrosis marker (Figure 2). The study is still recruiting patients at the National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia, from June 27th, 2019.

Study Population

Patients with rheumatic mitral valve stenosis (RMS) who undergo cardiac valve replacement in the National Cardiovascular Center Harapan Kita (NCCHK), Jakarta, Indonesia, will be screened for eligibility. The inclusion criteria of this study are patients with RMS or combined valve disease aged more than 18 years who undergo cardiac valve replacement operation with or without tricuspid valve repair. Patients must also have systolic blood pressure (SBP) ≥ 100 mmHg and diastolic blood pressure (DBP) ≥ 60 mmHg. The exclusion criteria of this study are patients with congenital heart disease, non mitral valve surgery, coronary artery bypass surgery or refusal to provide informed consent. Further exclusion criteria are adults aged 65 years or over, pregnant women, and patients with autoimmune disease, persistent hypotension (SBP < 100 mmHg), severe aortic stenosis (aortic valve orifice < 0.75 cm2), chronic renal dysfunction with serum creatinine > 2.5 mg/dL, or known ACEI intolerance. Participants who meet the criteria and are willing to join the RamiRHeD trial will be informed in detail about the study and will be required to sign the informed consent.

Outcomes

The primary outcomes of this study are the ST2 expression in mitral valve tissue and papillary muscle, and the secondary outcomes are soluble plasma ST2, clinical signs and symptoms that will be measured with the classification of NYHA (New York heart Failure Association), echocardiography results of: ejection fraction, TAPSE (Tricuspid Annular plane systolic excursion), End diastolic dimension, End systolic dimension, Mitral valve area, Mitral valve gradient, Tricuspid maximal velocity (Vmax), and Tricuspid regurgitation severity, as well as

laboratory test results for NT-proBNP concentration. Study participants will be followed up for cardiac and all-cause mortality outcomes until 1 year after the surgery.

Sample Size and Randomisation

This is a pioneering study analysing the effects of 5 mg ramipril on ST2 expression in mitral valve tissue in humans. A previous study that used ST2 human tissue was conducted by Marzullo et al in 2016[12]. The used carotid tissue from carotid endarterectomy, with a sample size of 41 consecutive patients. Because our study will use human tissue samples, we approached the sample size calculation using the multistage non-finite population method, using this specified precision estimation formula[13]: $N = (Z\delta)/E$, with N= sample size; $Z_{0.95}$= 1.96; δ N(0,1) = 1; and E= 0.05 for a 0.95 confidence interval. Therefore, we calculated a required sample of 1.65(1)/0.05= 33 samples.

According to the sample size of the previous study that analysed ST in human tissue and a sample size formula that is commonly used in *in vivo* studies, we decided to use a sample size of 30 for each arm, and with the addition of a drop out rate of 10%, this became total of 66 for the 2 arms.

The number includes a 10% dropout and withdrawal from each group. Randomisation will be done with an equal ratio of ramipril to placebo. A computerised sequence generator is used for randomisation. It will be linked with codes for placebo and treatment tablets provided by the manufacturer that was contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule will be identical between the two groups and will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.

Research technique

The mitral valve surgery (MVS) will be mitral valve replacement. Echocardiography will establish the diagnosis of rheumatic mitral valve disease. Rheumatic valve disease will be diagnosed with World Heart Federation Criteria (2012) for RHD[14]. The reference measurement for valve area is planimetry by two-dimensional echocardiography. The Doppler technique is used to assess the mean mitral gradient. Seller's classification on left ventriculography in a right anterior oblique view angle of 30° will be performed to evaluate the severity of mitral valve regurgitation. In cases of missing data, substitution measurements will be used as previously described: Doppler half time pressure for valve area and colour Doppler for mitral regurgitation.[14]

Patient classification and diagnosis of rheumatic mitral stenosis will be determined by qualified cardiologists, and the decision to perform mitral valve replacement surgery will be based on the consensus of the multidisciplinary team, consisting of cardiologists and cardiothoracic surgeons. Echocardiography will be performed by echocardiography-consultant cardiologists. Blood samples will be collected by trained nurses specialised in pathology clinic laboratory work. Biomedical analysts will be in charge of the analysis and collection of ST2 in plasma and mitral valves. Detailed interviews with the study participants will be done by a well-trained medical doctor. The data will come from questionnaires, laboratory tests, echocardiography, and biochemical tests. The study instruments will use the same technique, same tools, same brands, and same place for data collection from each study participant.

Pre-existing atrial fibrillation, left atrial size, concomitant rheumatic valve disease, NYHA class, and other clinical data and echocardiographic data will be documented before and after surgery and will be analysed by multivariate analysis.

Intervention

Daily capsules containing 5 mg ramipril or placebo to be taken orally will be provided for the study participants. An initial dose of 2.5 mg of ramipril will be given to the patients in the intervention group. If there are no significant adverse effects documented in the first 2 weeks after the initial dose, 5 mg of ramipril will be given in the subsequent weeks until 5 days before mitral valve replacement surgery. Participants will remain under the care of the treating cardiologist team. The routine medications of each patient will be continued. Capsules containing 5 mg ramipril or placebo will be given for a minimum of 3 months, up until 5 days before the mitral valve replacement.

Withdrawal and Drop out

Participants will be informed that they are able to withdraw from the study at any time and will sign a form stating this. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accordance with the consent obtained at trial entry. Drop-out criteria will be loss to follow-up, severe adverse events, and mortality due to any cause.

Sample Collection and Measurements

Clinical signs and symptoms will be documented before and after the study. Blood samples will be collected twice: before the intervention and one day before the mitral valve surgery. The routine blood analysis will include haemoglobin, platelet count, leucocyte count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Total cholesterol, random blood glucose, HbA1C, urea, creatinine, serum electrolytes, NTproBNP, and plasma ST2 will be determined. Echocardiography before the intervention and before surgery will be performed. Mitral valve tissue expression of ST2 will be measured by immunohistochemistry. Plasma ST2 will be measured using an enzyme-linked immunoabsorbent assay (ELISA) kit with the human ST2/IL33R antibody (R&D Systems, catalogue number DST200). This assay uses the technique of the quantitative sandwich enzyme immunoassay. A monoclonal antibody specific for human ST2 is pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any ST2 present is bound by the immobilised antibody. Unbound substances are washed away and then, an enzyme-linked polyclonal antibody specific for human ST2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells, and colour develops in proportion to the amount of ST2 bound in the initial step. After the colour development is stopped, the colour intensity is measured.

Mitral valve and papillary muscle tissue will be collected during mitral valve replacement surgery and will be saved in a sterile container filled with 10% formalin. ST2 expression will be observed using immunohistochemistry (IHC). Cross-linking chemicals, such as paraformaldehyde and glutaraldehyde, will be used to preserve the cellular structure. The fixation begins when the tissue is harvested. Tissue blocking is performed afterwards by placing the tissue sample in hot parafilm, after which it is put into a mould until hard. Following

fixation, tissue sections are obtained using a microtome. Decloaking methods consisting of heat and pressure treatment, enzyme digestion, and microwaving are done afterwards. Following decloaking, the parafilm on the slides is removed by baking, and then the IHC staining process can be started. The primary antibody is a monoclonal ST2 antibody. The secondary antibody is conjugated by biotin. The blocking buffer includes BSA. The chromogen that will be used is 3,3'-diaminobenzidine (DAB). DAB oxidation is catalysed by horseradish peroxidase (HRP), after which it forms a brown precipitate, so ST2 expression can be visualised under a light microscope. The tissue will then be counterstained using haematoxylin-eosin staining, so the non-ST2-expressing cells can be visualised in bluish colour. A negative control will use haematoxylin-eosin staining only. Measurements of cells that express ST2 will be performed under a microscope. The date, tissue type, antibody dilution, tissue treatment, and magnification of the microscope will be documented. ST2-expressing cells will be counted by more than one professional.

Statistical Analysis

Continuous variables are expressed as mean±SD, and categorical variables are expressed as percentages. The $\chi 2$ test will be used to see the relationship between dichotomous variables, and Student's t-test will be used for continuous variables. Single-variable correlation analysis and multivariable linear regression analysis will be performed. A P value <0.05 is considered statistically significant. The analyses will be performed with SPSS for Windows.

Ethics and Dissemination

The ethics of this study were approved by the ethics committee of National Cardiovascular Center Harapan Kita (NCCHK), Jakarta, Indonesia, with ethical code LB.02.01/VII/286/KEP.009/2018. This study has been registered at clinicaltrials.gov with the identifier code NCT03991910.

DISCUSSION

This study is planning to recruit rheumatic mitral valve patients to be randomised to obtain capsules containing either ramipril 5 mg or placebo. Rheumatic mitral stenosis is the main presentation of RHD that leads to significant morbidity and mortality. Recurrent or persistent valvulitis with bicommisural fusion usually leads to mitral stenosis. Previous studies suggest that RHD is an autoimmune disease that is associated with cytokine activities. Inflammatory cytokines are key regulators of immune processes.[4] Immunologic reactions caused by autoreactive antibodies continuously cause chronic inflammation and valvular fibrosis, which can be detected by an increase in sST2, an emerging biomarker for cardiac fibrosis.[10,15,16]

IL-33 is the natural ligand of ST2 and is highly expressed in smooth muscles and airway epithelia.[17] An inflammatory state stimulates the upregulation of ST2 by some cells, such as keratinocytes and dermal fibroblasts, and mechanical strain upregulates ST2 in cardiac fibroblasts.[17,18] The soluble ST2 isoform is increased under inflammatory conditions such as sepsis, allergic asthma, trauma, and pulmonary diseases.[19–22] Its elevation is also documented in some heart conditions, such as aortic stenosis and congestive cardiomyopathy, and this elevation is associated with the risk of heart failure and death.[23–27] In this study,

plasma ST2 is considered an inflammatory and fibrotic biomarker of rheumatic mitral stenosis. Because plasma ST2 can also increase in various conditions unrelated to cardiac fibrosis, this study also measures the ST2 expression in mitral valve tissue. Plasma ST2 describes the amount of ST2 in the circulation, whereas mitral valve cells that express ST2 describe the amount of transmembrane ST2.

ACEIs are commonly administered as the treatment of heart failure due to valvular regurgitation. Its use in MS is still debatable because of its hypotensive effect. A prior study assessing the safety of ACEIs in MS patients showed that the ACEI enalapril was well tolerated and safe up to a dose of 10 mg bid.[11] ACEIs are presumed to have vasodilatory effects in obstructive lesions and will decrease systemic vascular resistance through arterial vasodilatation, thus increasing the transvalvular gradient. Their anti-remodelling effect is also well established, and their long-term use has also been proven to improve left ventricular ejection fraction (LVEF) in patients with systolic dysfunction.[28] Because a prior study[11] demonstrated the efficacy and the potential benefits of ACEIs in improving outcomes in MS patients, this study aims to confirm and investigate the possible pathological mechanism of those improvements. This study will assess the effect of 5 mg ramipril as a cardiac antifibrosis treatment in severe MS RHD patients. Their plasma ST2 concentrations will be compared. Plasma ST2 concentration will also be compared before and after several months of consuming 5 mg ramipril. There will be no healthy controls for this study because of ethical limitations in the acquisition of mitral valve tissue. Mitral valve tissue will be acquired during mitral valve surgery. The expression of ST2 in mitral valve tissue will then be calculated semi-quantitatively and compared with the plasma ST2 results. It is hypothesised that ramipril will suppress the expression of ST2 in the cardiac mitral valve in patients with RHD.

In addition to the plasma ST2 level and the ST2 expression in mitral valve tissue, this study also compares the pre-post effects of 5 mg ramipril versus placebo on NT-proBNP concentration echocardiography strain parameters and clinical outcomes. Clinical signs and symptoms and echocardiography parameters have been evaluated in some studies of mitral valve stenosis, and showed that these were positively correlated with the NT-proBNP concentration.[29,30] This study will also compare the NT-proBNP concentration between patients receiving ramipril and placebo. We will also calculate the correlation between the NT-proBNP concentration and the ST2 plasma concentration and mitral valve expression.

Figure legends

Figure 1 Hypothesis.

Molecular mimicry is a defense mechanism of group A streptococcus to avoid immune cells. This mechanism allows immune cells to generate autoimmunity against protein the lining of endothelial cells and causing chronic inflammation and valvular damage. Continous proccess of chronic inflammation leads to valvular thickening and fibrosis, which is mediated by the Angiotensin II. Angiotensin II increase TGF-β expression and cause IL-33 to bind with sST2, and subsequently cause damage and fibrosis to the valvular tissue evenmore, which later will ended with rheumatic heart failure. ACEI is hypothized to counteract these processes by decreasing Angiotensin II conversion from Angiotensin I.

Figure 2 Research Flowchart

Authors contributions

Conception and design of the work was initiated by AMA, BS, AS, BR, and BD. AMA, BD, ES, and FT contributed to the acquisition, analysis, and interpretation of data for the work. This manuscript was drafted by AMA, BD, and ES. AMA, PD, AW, and MJC critically revised the manuscript. The author and coauthors gave final approval and agree to be accountable for all aspects of the work and ensuring its integrity and accuracy.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Patient and Public Involvement statement

Patients are not involved in the recruitment to and conduct of this study protocol.

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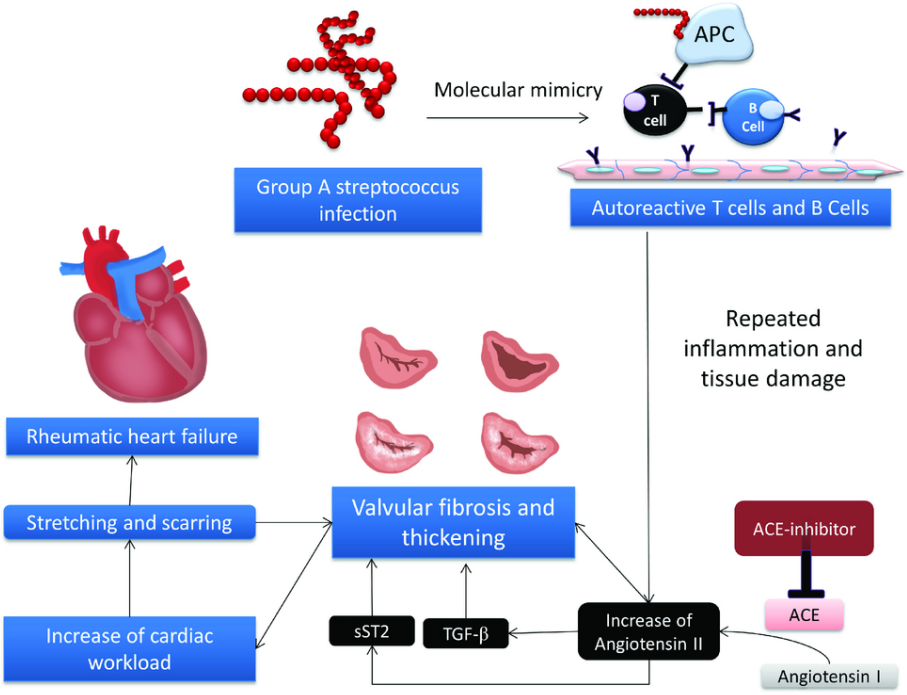

Figure 1 Hypothesis

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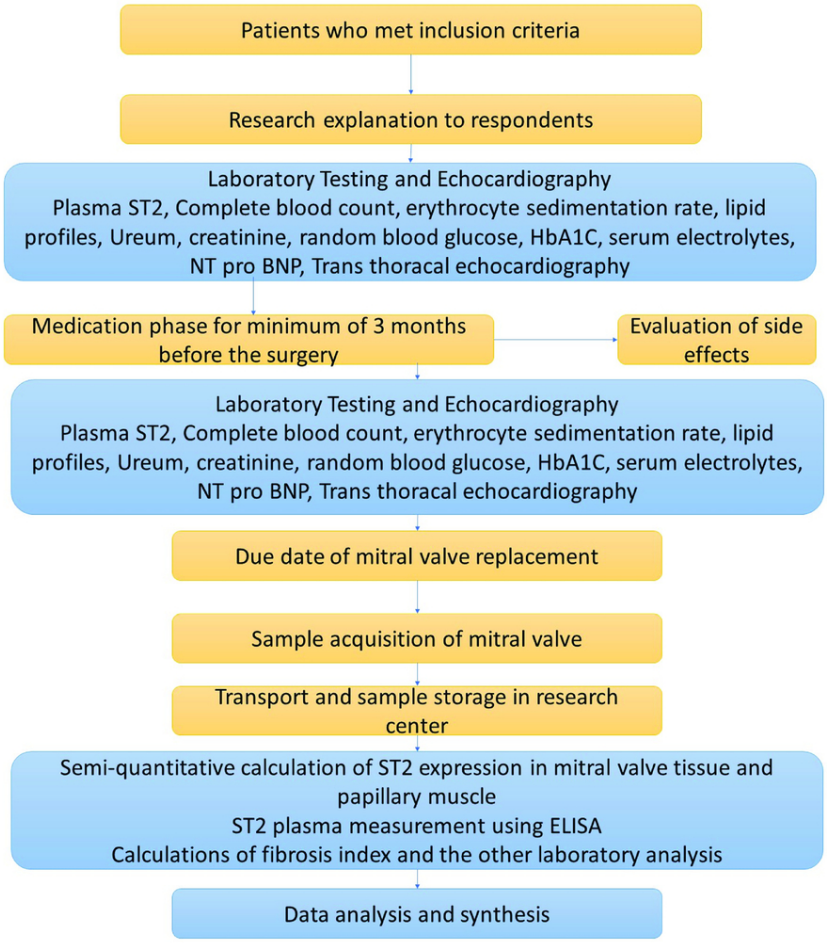


Figure 2 Research Flowchart

90x90mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym Randomised Controlled Trial into the role of ramipril in fibrosis reduction in RHD: The RamiRHeD trial protocol
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry Clinicaltrials.gov NCT03991910

2b All items from the World Health Organization Trial Registration Data Set

Register	ClinicalTrials.gov
Main ID	NCT03991910
Protocol ID	RamiRHeD
Date of Registration	19/06/2019
Prospective Registration	Yes
Primary sponsor	Harapan Kita National Cardiovascular Center/Indonesia University
Public title	The Effect of Ramipril in Suppressing ST2 Expression in Rheumatic Mitral Stenosis Patients
Scientific title	The Effect of Ramipril in Suppressing Gene Expression of Fibrosis in Cardiac Mitral Stenosis in Patients With Rheumatic Heart Disease
Date of first enrloment	June 27, 2019
Target sample size	66
Recruitment status	Recruiting
Study type	Randomised clinical trial
Study design	Allocation: Randomized. Intervention model: Parallel Assignment. Primary purpose: Treatment. Masking: Double (Participant, Investigator).
Phase	Phase 3
Countries of Recruitment	Indonesia
Health condition	ACE inhibitor Fibrosis; heart Mitral stenosis Rheumatic heart disease Rheumatic mitral stenosis
Intervention (s)	Drug: placebos Drug: Ramipril 5 mg oral capsule
Primary outcome	ST2 expression in mitral valve tissue
Secondary outcome	ST2 plasma level NT-proBNP concentration (pg/ml) Ejection fraction TAPSE (tricuspid annular plane systolic excursion NYHA class

Protocol version 3 Date and version identifier

Released: 19-06-2019

Funding 4 Sources and types of financial, material, and other support

No specific fundings outside our institution (Harapan Kita National Cardiovascular Center, Indonesia)

Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors Ade M. Ambari • Budhi Setianto• Anwar Santoso• Basuni Radi • Bambang Dwiputra Department of Cardiology and Vascular Medicine Faculty of Medicine University of Indonesia, National Cardiovascular Center Harapan Kita, Jakarta, Indonesia Eliana Susilowati •Fadilla Tulrahmi Research Assistant Division of Preventive and Rehabilitative Cardiology, National Cardiovascular Centre Harapan Kita, Jakarta, Indonesia Maarten .J.M. Cramer • P.A. Doevendans • Annemiek Wind Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands Role: Ade M. Ambari conceived the study. Ade M. Ambari, Pieter A. Doevendans, Maarten .J.M. Cramer, Budhi Setianto, Anwar Santoso, Basuni Radi, Bambang Dwiputra initiated the study design and conceptual framework. Ade M. Ambari, Bambang Dwiputra contribute in patients' recruitment and assessment,writing and editing. Eliana Susilowati contribute to the biomedical methods, sample collection, writing and editing. Fadilla Tulrahmi conducting the primary statistical analysis, randomisation, and sample size calculation. Ade M. Ambari, Pieter A. Doevendans, Maarten .J.M. Cramer, Annemiek Wind contribute to writing, protocol editing, language editing. All authors contributed to refinement of the study protocol and approved the final manuscript.
	5b	Name and contact information for the trial sponsor Trial sponsor: Harapan Kita National Cardiovascular Center, Jakarta Contact name: Ade Meidian Ambari Address: S Parman Street number 87, Special Region of Jakarta, Indonesia Telephone: 0215681111 Email: dr_ade_meidian@yahoo.co.id
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

Introduction

Principal Investigators
Design and conduct of RCTRHDMS
Preparation of protocol and revisions
Organising steering committee meetings
Publication of study reports
Members of TMC [*Trial Management Committee*]

Steering committee (SC)
Agreement of final protocol
All lead investigators will be steering committee members
Reviewing progress of study and if necessary agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study.

Trial Management Committee (TMC)
(Principle [*sic*] investigator, Research Physician, Administrator)
Study planning
Organisation of steering committee meetings
Provide annual risk report to ethics committee
report serious adverse events (SAE) to medical committee and ethics committee
Responsible for trial master file
Budget administration and contractual issues with individual centres
Advice for lead investigators
Audit of 6 monthly feedback forms and decide when site visit to occur.
Assistance with international review, board/independent ethics committee applications
Data verification
Randomisation
Organisation of central serum sample collection

Data Manager
Maintenance of trial IT system and data entry
Data verification

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention

Rheumatic heart disease (RHD) is a major burden in developing countries home to 80% of all people living with the disease where it causes most of the cardiovascular morbidity and mortality in children and young adults. Chronic inflammation and fibrosis of heart valve tissue due to chronic inflammation in RHD will cause calcification and thickening of the impacted heart valves, especially the mitral valve. This fibrogenesis is enhanced by the production of Angiotensin II by increasing TGF-β expression and latter, the binding of IL-33 which is known to have anti-hyperthropic and anti-fibrotic effects to sST2. Its binding to the non-natural ligand of sST2 will worsen the fibrosis. Therefore, we hypothesized that Angiotensin-converting enzyme inhibitor (ACEI) will improve rheumatic mitral valve stenosis.

Existing knowledge: Angiotensin-converting enzyme inhibitors (ACEI) are first-line therapy in cardiac failure, and their symptomatic and survival benefits extend beyond afterload reduction. Reduction in fibrosis and anti-proliferative and neurohumoral effects contribute to the ACEI effect that is not reproduced by pure vasodilators. ACEI was well tolerated in symptomatic RHD associated with significant mitral stenosis and preserved left the ventricular systolic function. New therapies targeting ST 2 and their receptors as studied in some autoimmune diseases may promise a new approach for patients with RHD. We are assessing the effect of Ramipril in suppressing fibrotic protein expression in mitral valve (measured with ST2 expression) of patients with RHD in the National Cardiac Center Harapan Kita hospital Jakarta Indonesia.

Dose selection: Prevoious study (SCOPE trial) showed that ACEI (Enalapril) was well tolerated in symptomatic RHD associated with significant aortic and/or mitral stenosis and preserved left ventricular systolic function until dose of 10 mg bid. This study use Ramipril 5 mg as it is more commonly used in Indonesia.

Need for a trial: Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. In developing countries, percutaneous balloon mitral valvuloplasty and mitral valve surgery are the therapeutic options for rheumatic mitral stenosis. Both of these treatments involve enormous expenses; it is the public health cost burden for developing countries. Valvular anti-inflammatory and anti-fibrosis medical therapy to suppress the progression of the disease is needed in rheumatic mitral stenosis patients.

	6b	Explanation for choice of comparators Current guidelines for valvular intervention do not include ACEI as therapy in rheumatic mitral stenosis patients. This study is divided in 2 arms. The first arm will be given Ramipril 5 mg as the intervention, while the second arm, will be given placebo as the comparator. The other individualized treatments for rheumatic valvular disease was still given in both armsas indicated.
Objectives	7	Specific objectives or hypotheses The investigators hypotized that administration of Ramipril 5 mg for 3 months will reduce expression of ST2 as fibrosis biomarkers, in the cardiac mitral valve of patients with Rheumatic Heart Disease with mitral stenosis.
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) This is a single-centre, double-blind, placebo-controlled, pre-post test design, randomised clinical trial. Patients with mitral stenosis valvular dysfunction due to rheumatic process planned for cardiac valve replacement were given Ramipril 5 mg or placebo for minimum 12 weeks before the surgery. ST2 was checked as fibrosis marker. This study will be conducted in the Department of Cardiology and Vascular Medicine, University Indonesia, National Cardiac Center Harapan Kita Hospital, Jakarta, Indonesia from June, 27th 2019.

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained Study setting: in a national academic hospital. Patients come from various regions in one country (Indonesia).
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) • Inclusion criteria of this study are: Patients with mitral valve stenosis or a combination • aged more than 18 years • undergo cardiac valve replacement operation with or without a tricuspid valve repair, • patients with systolic blood pressure (SBP) ≥ 100 mmHg and diastolic blood pressure (DBP) ≥ 60 mmHg • passed in medication phase without side effect minimum 4 weeks until operation schedule

Interventions

11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered

Arms and Interventions

Arms	Interventions
Placebo Comparator: control control patients will be given a placebo	Drug: Placebos the control group will be given placebo inside a capsule, so study participant won't be able to know the drug and doses inside the capsule (for masking)
Experimental: treatment Ramipril 5 mg treatment group	Drug: Ramipril 5Mg Oral Capsule the treatment group will be given Ramipril 5 mg inside a capsule, so study participant won't be able to know the drug and doses inside the capsule (for masking)

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)

Participants will be informed that they are able to withdraw from the study at any time and will sign a reference as proof. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry. Drop out criteria will be Participants who are lost to follow up, participants with severe adverse events, mortality due to any cause.

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)

Patients' adherence will be monitored with the drug return. Patients will be contacted via phone number to be reminded for routine administration. Patients are provided with the investigator contact if needed something to ask or to report adverse events or side effects (if any). Patients will be asked to visit the hospital where this study is enrolled to meet the investigator monthly. Each month, capsule return will be counted and patient will also be evaluated for the symptoms and vital signs.

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial

Rescue medication: stop the drug. Patient will be admitted to hospital and undergone treatments according to the adverse effects.

Prohibited concomitant medication: Angiotensin receptor blocker.

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended

Primary Outcome Measures :

- ST2 plasma level

plasma level of ST2 measured by ELISA

Secondary Outcome Measures :

- ST2 expression in mitral valve tissue

expression of ST2 in mitral valve tissue, using immunohistochemistry method

- NT-proBNP concentration (pg/ml)

concentration of NT-proBNP, plasma markers for cardiac dysfunction.

- ejection fraction

echocardiography parameter

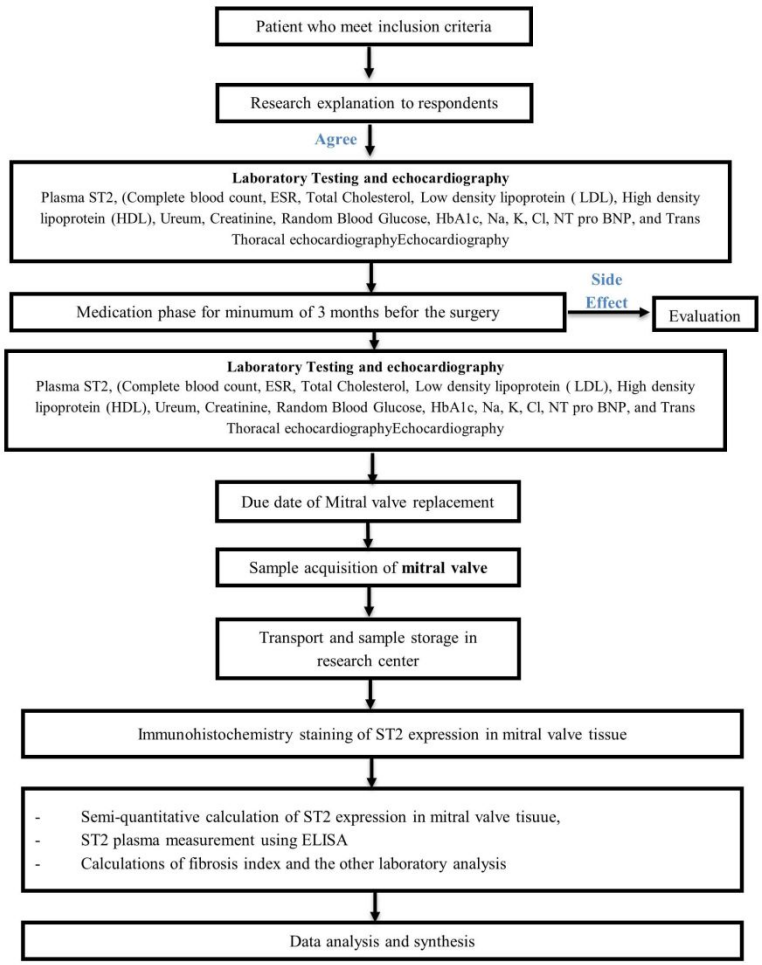
- TAPSE (tricuspid annular plane systolic excursion)

echocardiography parameter to asses right ventricular function

- NYHA class

related symptoms will be graded in class I to IV according to NYHA.

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)



Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations This is a pioneer study for analysing effects of Ramipril 5 mg toward ST2 expresion in mitral valve tissue in human. Previous study that use ST2 human tissue is a study from Marzullo et at in 2016[12] that use carotid tissue from carotid endarterectomy, with sample size of 41 consecutive patients. Because our study will use human tissue sample, we approached the sample size calculation using multistage non-finite population method, using this specified precision estimation formula[13]: $N = (Z\delta)/E$, with N= sample size; $Z_{0.95}$= 1.96; δ N(0,1) = 1; and E= 0.05 for 0.95 confidence interval. So we calculate the sample and found that 1.65(1)/0.05= 33 samples. According to the sample size of the previous study that analyse ST in human tissue and a sample size formula that commonly used in in-vivo study, we decide to use a sample size of 30 for each arm, and with the addition of drop out rate of 10%, become total of 66 for 2 arms. The number includes a 10% dropout and withdrawal in each group. Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size Patient recruitment will involves several cardiologists and cardiothoracic surgeons. All cardiologists and cardiothiracic surgeons in the hospital will be informed about this study, and to inform back if their patient suffered from Mitral valve stenosis. Technical meetings will be held and the profesionals related to this study will be invited.

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned Randomisation will be done in an equal ratio of Ramipril to placebo. An online, web-based sequence generator system will be used. It will be linked with codes for placebo and treatment tablets provided by the manufacturer contracted to produce the trial medication. Researchers and participants will be blinded. After randomisation, the treatment pack and capsule are identical between both groups will contain either active tablets or placebo. The principal investigator will have no access to the randomisation list.
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions Allocation sequence generation are from online, web-based sequence generator system. Participants will be enrolled with staff member responsible for patients enrollment.
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how Trial participants, investigators, analysts, care providers, will be blinded. Reserach assisstants whose role are to follow-up, evaluate and monitor the patients condition will not be blinded, so the drug could be stopped easily if there is any adverse effect from the treatment group.
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial Under the circumstances where actual treatment is absolutely necessary for further management of the patient, unblinding is permissible.

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol Data collections will involve profesional in each filed of data collection. For example: patients will be diagnosed with experienced cardiologists, surgery desicion based on the decision of a multidisciplinary team, echocardiography will be performed by the cardiologist who is specialized in echocardiography. Blood samples will be drawn by the experienced nurses in pathology clinic laboratory, ST2 plasma and tissue will be collected with the biomedical anlalysts, interview of study participants will be done by the trained assessors which. Study instruments involve questionnaires, laboratory tests, echocardiography, and biochemical tests. Study instruments will use same technique, same tools, same brands, and same place for data collection of each study participant.
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols Participants will be informed that they are able to withdraw from the study at any time and will sign a reference as proof. They will be informed that this will not affect their clinical care. Basic clinical data and samples already collected will be included in the analyses in accord with the consent obtained at trial entry.
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol Participant files will be stored in numerical order and stored in a secure and accessible place and manner. Participants data will be copied in softfile and have back-up data.

Statistical methods 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol

Variable/Outcome	Hypothesis	Outcome Measure	Methods of Analysis
1) <u>Primary</u>	Participants adherence of Ramipril 5 mg for 3-6 months or placebo 3-6 months will be 80%	Remaining drug in the participants is not more than 20% of the study drug given, to evaluate the adherence.	Manual counting, univariate analysis
a) ST2 expression in mitral valve tissue	Reduction occurr	Mitral valve tissue ST2 measured using percentage of-cell-expressing ST2 using immunohistochemistry method	T-test
2) <u>Secondary</u>			
a) ST2 plasma level	Reduction occurr	Plasma ST2 concentration will be measured with the concentration calculated according to the absorbance in ELISA technique	T-test
b) NT-proBNP concentration	Reduction occur	NT-proBNP concentration is measured in clinical pathology laboratorium using ELISA.	T-test
c) Ejection fraction(EF) and TAPSE	Improvement occurr	Measure was performed using same echocargiography pre and post test, documented in percentage.	T-test
d) NYHA class	Reduction occurr	NYHA class will be measured accoding to the clinical signs and symptoms classified in NYHA	Chi-square test

20b Methods for any additional analyses (eg, subgroup and adjusted analyses)

Continuous variables were expressed as mean±SD and categorical variables as percentages. The $\chi 2$ test was used to see the relationship between dichotomous variables and the Student t-test for continuous variables. Single variable correlation analysis and multivariable linear regression analysis will be performed. A P value <0.05 was considered statistically significant. The analyses were performed with the use of SPSS for Windows.

20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

This study will be analysed in per-protocol fashion. Handling missing data will use multiple imputation method. Analysis of the primary endpoint will be based on a log-rank test and, therefore, not affected by patient withdrawals (as they will be censored) provided that dropping out is unrelated to prognosis. The effect that any missing data might have on results will be assessed via sensitivity analysis of augmented data sets. Dropouts (essentially, participants who withdraw consent for continued follow-up) will be included in the analysis by modern imputation methods for missing data. After the imputations are completed, all of the data (complete and imputed) will be combined and the analysis performed for each imputed-and-completed dataset. Rubin's method of multiple imputation will be used to estimate treatment effect.

Methods: Monitoring

Data monitoring

21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

A Data Monitoring Committee (DMC) has been established. The DMC is independent of the study sponsor. During the period of recruitment to the study, interim analyses will be supplied, in strict confidence, to the DMC, together with any other analyses that the committee may request.

21b Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial

An interim-analysis is performed on the primary endpoint when 80% of patients have been randomised and mitral valves have been obtained. The interim-analysis is performed by a statistician in this study member. Stopping decision (if needed) based on the desicion of the primary investigators and will be reported to the ethics commitee.

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct Adverse events that happen before the patient started to receive study intervention will not be reported as it is not related to the study drug. All adverse events occurring after the patient receiving study intervention until the end of the study will be recorded. Serious adverse event (SAE) related to this study treatment will be reported to the institutional review board and ethical committee. Serious adverse event including: life-threatening condition with immediate risk of death, severe or permanent disability, prolonged hospitalisation, or a sgnificant hazard determined by the data safety monitoring board. SAE that is believed by the investigator and medical committee to be causally related to the study drug will be reported. SAE occuring a month after the subject is discontinued from the study will not be reported unless the investigators believed that the event have been caused by the study drug. The causal effects will be determined according to the temporal relationship, clinical course, previous medical conditions and concomitant medications.
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor Ethic committee will audit the trial conduct

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval This protocol and the template informed consent forms contained in Appendix I has been reviewed and approved by the IRBs/ECs [*institutional review boards/ethical committees*] with respect to scientific content and compliance with applicable research and human subjects regulations. The protocol, site-specific informed consent forms is in local language. Participant education and recruitment materials, and other requested documents and any subsequent modifications also will be reviewed and approved by the ethical review bodies (IRBs/ECs).

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. If such amandement happens, it will request an approval of the Ethics Committee/IRB [*institutional review board*] prior to implementation.
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) The member of investigators will explain and obtain informed consent.
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable Additional biological samples will be obtained to be stored for use in future studies. It will be stored in research center inside this hospital with adequate and certified samples handling. A materials consent will be obtained to the collection of the plasma speciments.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial All study-related information will be stored securely in the principal investigator's locker with limited access. All participants data will be identified by a coded ID number to maintain the confidentiality. Laboratory speciments will be stored in research center with a safe storage and will be identified also with coded ID. Local database will be secured with password access systems and the access will be limited.
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site All of the investigators disclose no conflict of interests.
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators Data management coordinating team will oversee the intra-study data sharing process. All principal investigators will be given access to the cleaned datasets. Principal investigators will have direct access to their own data sets and will have access to other sites data by request. To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation Study participants are covered by compensation for negligent harm through the standard of this hospital. This will include cover for additional health care, if it has causal relationship with the study drug.
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions Investigators is not expected to report the data as individual report. All presentations and publications are expected to protect the integrity of the major objectives of the study; data that break the blind will not be presented prior to the release of the main results. Recommendation as to the timing of presentation of endpoint data which they might be presented will be given by the steering committee. Each paper abstract must be submitted to the appropriate subcommittee for review of its appropriateness and scientific merit prior to submission, the subcommittee may recomend changes to the authors and submit its recommendations to be approved by the steering committee. Publications of papers to workshops, symposia, volumes etc will be in the right of the principal investigators, and principal investigators could appoint and give permission for the other investigators or other party to present this paper. the study results will be released to the participating physicians, patients, and general medical community.
	31b	Authorship eligibility guidelines and any intended use of professional writers Topics suggested for presentation or publication will be circulated to the PIs [*Principal investigators*] of the CCCs [*Core Coordinating Centers*], the DCC [*Data Coordinating Center*], and research center in hospital. These groups are requested to suggest and justify names for authors to be reviewed by the PC [*Publications Committee*].
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code Data sharing statement: no later than 5 years after the collection of the 1-year post randomisation interviews, we will deliver a completely deidentified data set to an appropriate data archive for sharing purposes.

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates

RAHASIA 1

Lembar Informasi dan Persetujuan Pasien

Judul Penelitian :

The Effect of Ramipril in Suppressing Gene Expression of Fibrosis in Left Atrial Appendage in Cardiac Mitral Stenosis Rheumatic Heart Disease Patients

Latar Belakang dan Tujuan Penelitian

Penyakit jantung rematik merupakan beban penyakit utama di negara berkembang yang hampir 80% seluruh orang hidup dengan penyakit ini, dimana sebagai penyebab tingginya angka kematian dan kesakitan kardiovaskular pada anak dan remaja. Berdasarkan *Global Burden of Disease Study* (2010), jumlah pasien yang mengalami disabilitas karena penyakit jantung rematik sebanyak 10,1 juta per tahun di seluruh dunia.[1] *Rheumatic Mitral Valve Stenosis* (RMS) merupakan indikator utama penyakit jantung rematik yang dapat berdampak signifikan pada kematian dan kesakitan.

ST2 merupakan bagian reseptor IL-1 yang terdiri dari 2 bentuk, *a trans-membrane receptor* (ST2L) dan *soluble decoy receptor* (sST2).[4] Keduanya diinduksi di *cardiomyocyes* dan *fibroblast* yang terpapar tekanan biomekanik. Fungsi dari ST2 pada penyakit kardiovaskular, IL-33 telah terbukti memiliki efek anti-hipertrofik dan anti-fibrotik pada jantung, ditransduksi oleh ST2L.[4]

Angiotensin-Converting Enzyme Inhibitors (ACEI) sering digunakan untuk mencegah dan mengobati gagal jantung karena penyakit katup regurgitasi. Mayoritas pasien dengan penyakit jantung rematik simtomatik (RHD) memiliki mitral stenosis (MS) yang signifikan dan menolak terapi ACEI, hal ini disebabkan karena ditakutkan adanya hipotensi di kemudian hari dengan adanya obstruksi tetap.[5] ACEI pula dapat ditoleransi pada penyakit jantung rematik simtomatik berkaitan dengan mitral stenosis yang signifikan dan tetap mempertahankan fungsi sistolik ventrikel kiri.[5]

Efikasi pencegahan sekunder terbatas dalam mencegah progresivitas penyakit jantung rematik sehingga diperlukan adanya strategi dan terapi yang dibutuhkan untuk mencegah hal tersebut.[3] Terapi terbaru menargetkan ST2 dan reseptor seperti yang diteliti pada penyakit autoimun memungkingkan adanya pendekatan baru untuk pasien penyakit jantung rematik. ACEI merupakan agen dengan efek anti fibrosis. Oleh karena itu, peneliti ingin mengetahui efek Ramipril dalam memodulasi ekpresi gen fibrosis pada

jaringan katup dan appendiks atrium kiri pasien dengan penyakit jantung rematik di RS Jantung dan Pembuluh Darah Harapan Kita.

Penjelasan tentang Prosedur Pelaksanaan

Pasien dengan penyakit jantung rematik mitral stenosis yang akan dilakukan tindakan MVR/r akan diberikan Ramipril atau Placebo selama minimal 3 bulan dan maksimal 6 bulan yang akan dikonsumsi setiap hari sampai waktu untuk dilakukannya tindakan operasi.

Partisipasi sukarela/pengunduran diri

Partisipasi anda dalam penelitian ini bersifat sukarela, anda dapat menolak untuk berpartisipasi. Jika anda memutuskan untuk berpartisipasi, maka anda akan diberi lembar informasi ini untuk dipelajari isi dan tujuan penelitian. Anda dapat menyimpan lembar informasi ini dan anda akan diminta untuk mengisi dan menandatangani formulir persetujuan ini.

Anda dapat mengundurkan diri di awal saat anda membaca informasi dari penelitian ini dan tidak menyetujui untuk mengikuti penelitian tanpa harus memberikan alasan. Pengunduran diri anda tidak menimbulkan sanksi apapun dan anda tidak akan kehilangan manfaat yang akan menjadi hak anda.

Manfaat partisipasi

Secara pribadi anda dapat mengambil manfaat berpartisipasi dalam penelitian ini karena dapat membantu mengurangi gejala dan tanda penyakit jantung katup mitral rematik. Anda juga dapat memberikan manfaat bagi orang lain dalam pengembangan ilmu pengetahuan dan peningkatan kesehatan masyarakat secara luas.

Resiko dan Ketidaknyamanan

Konsekuensi dari partisipasi ini mengharuskan anda untuk minum obat maksimal selama 6 bulan setiap hari sebelum dilakukannya tindakan MVR/r. Partisipan akan melakukan pengambilan obat setiap bulannya ke tempat penelitian. Selain itu juga akan menjalani beberapa pemeriksaan penunjang medis seperti echocardiography dan

pengambilan darah yang akan dilakukan sebelum fase minum obat, dan setelah selesai minum obat.

Kerahasiaan

Semua data pada penelitian ini akan diambil tanpa memberikan identitas anda. Kerahasiaan data dan identitas Anda dilindungi oleh hukum dan atau peraturan yang berlaku, dan tidak akan diberitakan secara umum. Pada saat hasil diumumkan, identitas Anda akan tetap terjaga kerahasiaannya.

Hanya pihak yang terlibat dalam penelitian ini saja yang akan diberikan wewenang untuk dapat memperoleh dan mengetahui keadaan kesehatan Anda, termasuk didalamnya dokter Anda dan perawat, rumah sakit, pihak sponsor dan perwakilannya, dan atau anggota dari Komisi Etik. Anda mempunyai hak untuk mendapatkan segala informasi yang berhubungan dengan keikutsertaan Anda dalam penelitian ini.

Persetujuan Komite Etik Kedokteran

Penelitian ini diteliti dan disetujui oleh Komisi Etik Pusat Jantung Nasional Harapan Kita.

Biaya

Partisipan yang mengikuti penelitian ini akan diberikan biaya perjalanan/transportasi dari rumah ke tempat penelitian sebesar Rp. 100.000,- pada setiap bulan selama masa konsumsi obat. Biaya penelitian ditanggung oleh peneliti yang termasuk dalam paket penelitian.

Lain-lain

Jika anda merasa tidak nyaman, anda dapat memilih untuk tidak ikut serta dalam penelitian. Hal ini tidak akan mempengaruhi pelayanan rumah sakit terhadap anda di masa mendatang

Penawaran untuk Menjawab Pertanyaan

Jika Anda mempunyai pertanyaan-pertanyaan mengenai studi ini, Anda dapat menghubungi:

dr. Ade Meidian Ambari, SpJP(K)
No. telepon. 021 – 568 4085 ext 2209

Silahkan untuk tidak mendatangani formulir ini jika anda tdak mempunyai kesempatan untuk bertanya atau tidak menerima jawaban-jawaban yang memuaskan terhadap pertanyaan-pertanyaan anda.

Pernyataan Persetujuan

Dengan menandatangani formulir ini, saya menyetujui bahwa penelitian ini telah dijelaskan kepada saya dan semua pertanyaan saya telah dijawab dengan memuaskan. Saya juga mempunyai hak untuk dapat mengundurkan diri dari penelitian ini setiap saat. Dengan pengertian tersebut, saya dengan sukarela ikut serta dalam penelitian ini. Saya mengerti bahwa formulir ini akan disimpan bersama dengan data kesehatan saya dan saya akan mendapatkan *copy* dari formulir ini.

Nama Pasien : _____	Nama Wali : _____
Tanda tangan : _____	Tanda tangan : _____
Tanggal : ___/___/_____	Tanggal : ___/___/_____

Nama Dokter / Asisten : _____
Tanda tangan : _____
Tanggal : ___/___/_____

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable Plasma speciments will be stored in biobank inside the research center in this hospital, and has been approved by the medical and ethical committee for the possible further researches.

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.