## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Randomised Controlled Trial into the role of ramipril in fibrosis reduction in Rheumatic Heart Disease: The RamiRHeD trial protocol |
| **AUTHORS** | Ambari, Ade; Setianto, Budhi; Santoso, Anwar; Radi, Basuni; Dwiputra, Bambang; Susilowati, Eliana; Tulrahmi, Fadilla; Wind, Annemiek; cramer, maarten jan; Doevendans, Pieter |

## VERSION 1 – REVIEW

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| **REVIEWER** | Gajbhiye, Rahul<br>NIRRH, Department of Clinical Research |
| **REVIEW RETURNED** | 27-Feb-2021 |

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| **GENERAL COMMENTS** | Comments to Author<br>Plagiarism<br>Authors need to pay attention to the following text for Plagiarism.<br>• Page 4, line 9-15, 16-24, 53-60<br>• Page 5, Line 15-25<br>• Page 6, Line 46-57<br>• Page 7, Line 48-57<br>• Page 8. Line 44-55<br>Minor comments<br>The manuscript requires editing for grammar, spell check, typo errors etc. I have pointed out some of these errors.<br>• Spelling errors : Page 3, line 29, Anti-hyperthrophic<br>• Full stop to be replaced by comma, Page 4 line 13- 233.000<br>• The sentence is starting with small letter, Page 4 line 23, fibrogenesis.<br>• Page 5, line 15, valvular dibrosis<br>• Page 6, line 8, outcome<br>Following SPIRIT items are not addressed adequately.<br>Recruitment (15)<br>• The person who will identify patients (e.g. GP, surgeon, study nurse) is not specified<br>• Duration of recruitment is not specified in the methods<br>Allocation implementation (Item 16 c) is not clearly described. |

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| **REVIEWER** | Gupta, Prabha<br>Medical College, Cardiology |
| **REVIEW RETURNED** | 24-May-2021 |

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| **GENERAL COMMENTS** | This is a well planned study .Ramipril is likely to have more effects on the patients with mitral stenosis. Some patients would be in atrial fibrillation ,Ramipril would improve atrial remodeling and improve outcomes .High doses of Ramipril may not be tolerated in very tight mitral stenosis. So I suggest - the presence of absence of atrial fibrillation should be recorded specifically. The left atrial size, area |

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| | and volume before and after Ramipril should be noted. Further the NYHA class before and after Ramipril should be recorded. Ramipril 5 mg may not be tolerated so a dose of Ramipril 2.5 mg should be initiated and then stepped up if possible .The presence of mitral regurgitation and the grade of this ,and the presence of aortic regurgitation should be noted.(I think the authors have decided to record these points but these may give useful results .I routinely give a low dose of Ramipril to patients with atrial fibrillation and mitral stenosis with large left atria .The main sources of ST2 are cardiomyocytes and cardiac fibroblasts.So it would be prudent to try a staining of a mitral valve before initiating the study.Alternatively the papillary muscles if resected could be stained.There are references of ST2 receptors on the aortic valve and coronaries.<br>ST2 is a predictor of mortality and this should also be analysed.(But the number is not sufficient .)So the study could be made to include patients not going for surgery too. |

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| **REVIEWER** | Regazzoli, Damiano<br>IRCCS Humanitas Research Hospital |
| **REVIEW RETURNED** | 30-May-2021 |

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| **GENERAL COMMENTS** | While the study seems to be interesting, I have two major concerns:<br>1-It is not a contemporary study, at least for european countries<br>2-The results may be influenced by non-mitral surgery (I mean, that surgery that is not an exclusion criterium such as tricuspid or LAA procedures)<br>Furthermore, english grammar needs revisions.<br>I think that this protocol may be suitable for publication on a smaller or more specialistic Journal. |

## VERSION 1 – AUTHOR RESPONSE

B. Reviewer: 1
Dr. Rahul Gajbhiye, NIRRH
Comments to the Author:

Comments to Author

1. Plagiarism
Authors need to pay attention to the following text for Plagiarism.
• Page 4, line 9-15, 16-24, 53-60
• Page 5, Line 15-25
• Page 6, Line 46-57
• Page 7, Line 48-57
• Page 8. Line 44-55
2. Minor comments
The manuscript requires editing for grammar, spell check, typo errors etc. I have pointed out some of these errors.
• Spelling errors : Page 3, line 29, Anti-hyperthrophic
• Full stop to be replaced by comma, Page 4 line 13- 233.000
• The sentence is starting with small letter, Page 4 line 23, fibrogenesis.
• Page 5, line 15, valvular dibrosis
• Page 6, line 8, outcome
3. Following SPIRIT items are not addressed adequately.

Recruitment (15)
• The person who will identify patients (e.g. GP, surgeon, study nurse) is not specified
• Duration of recruitment is not specified in the methods
Allocation implementation (Item 16 c) is not clearly described.

Authors' responses:
Thank you for reminding us regarding the plagiarism issue, and that there are many overlapping text presented in our manuscript.
1. We paraphrased and revised the sentences as follows:
o Former Page 4, line 9-24 can be seen in Introduction section line 49-59:
"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]"
o Page 4, line 53-60 can be seen in introduction section lines 83-89:
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]
o Page 5, Line 15-25 become can be seen in Introduction section last paragraph line 98-106
"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)."
o Page 6, Line 46-57 can be seen in Methods section, research technique subsection, line 165-177:

"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."
o Page 7, Line 48-57 can be seen in methods section, Sample Collection and Measurements subsection, lines 220-227:
o "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. “

o Page 8. Line 44-55 can be seen in Discussion section lines 260-266:

“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]”

2. Typographical errors has been revised to the correct words and punctuations.

3. Thank you for your suggestions,

- The person who identify the patients has been specified in methods section lines 170-173:

“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. “

- There is no specific study time range for the recruitment duration, the recruitment will be ended after the sample size has been achieved. Allocation of intervention has been revised and more specified, as can be seen in methods section, intervention subsection, lines 185-192:

“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.”

- and in methods section, Sample Size and Randomisation subsection, lines 152-158:

“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.”

C. Reviewer: 2

Dr. Prabha Gupta, Medical College

Comments to the Author:

This is a well planned study .Ramipril is likely to have more effects on the patients with mitral stenosis. Some patients would be in atrial fibrillation ,Ramipril would improve atrial remodeling and improve outcomes .High doses of Ramipril may not be tolerated in very tight mitral stenosis.

1. So I suggest - the presence of absence of atrial fibrillation should be recorded specifically. The left atrial size, area and volume before and after Ramipril should be noted. Further the NYHA class before and after Ramipril should be recorded.

2. Ramipril 5 mg may not be tolerated so a dose of Ramipril 2.5 mg should be initiated and then stepped up if possible.

3. The presence of mitral regurgitation and the grade of this ,and the presence of aortic regurgitation

should be noted.(I think the authors have decided to record these points but these may give useful results .I routinely give a low dose of Ramipril to patients with atrial fibrillation and mitral stenosis with large left atria.
4. The main sources of ST2 are cardiomyocytes and cardiac fibroblasts. So it would be prudent to try a staining of a mitral valve before initiating the study. Alternatively the papillary muscles if resected could be stained. There are references of ST2 receptors on the aortic valve and coronaries.
5. ST2 is a predictor of mortality and this should also be analysed.(But the number is not sufficient .)So the study could be made to include patients not going for surgery too.
Authors' responses:
1. Thank you for your valuable suggestions, the presence of Atrial fibrillation prior to the study and prior to the surgery will be documented. Clinical parameters including NYHA, and also echocardiographic parameters such as LA size, area and volume will be recorded before the intervention and before the surgery (treatment cessation). We has revised and clarify about this circumstances in our manuscript, in methods section, research techniques subsection, lines 180-182:
"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."

2. Thank you for your concern of the possible intolerable dose of Ramipril 5mg. We revise our study flow by adding the initial dose trial period of 1 month with 2.5 mg of Ramipril before considering to continuation of Ramipril 5 mg. We revise this in methods section, lines 186-189:
"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."
3. Concomitant rheumatic valve disease or any valve repair will be documented and analysed using multivariate analysis whether they can interfere the outcomes, and could be the important sub-analysis in this study. Thank you for the highlights, we will document all patients' present and past medical histories, including any mitral regurgitation and or aortic regurgitation.
4. Staining mitral valve of a healthy individual could not be ethically performed. Mitral valves of rheumatic mitral stenosis undergoing mitral valve replacement in placebo group will hopefully could demonstrate the comparison of the ST2 expression. Papillary muscle resection could be requested to the cardio-thoracic surgeons, so we can analyse the ST2 expression in the resected papillary muscle besides the mitral valve, thank you for this valuable suggestion. We revise our text and add the information about papillary muscle in subsection of Sample Collection and Measurements in methods section, lines 218-219, as the following:
"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."
5. Thank you for the valuable suggestions, we will follow-up our study participants for the mortality outcome after the surgery. Idea of analysing the mortality in non-surgery patients could be our next research project because there will be a sample size discrepancy if it is included in this study. Mortality outcome has been added in methods section as the additional secondary outcome, as can be seen in methods section, outcomes subsection, lines136-137:
"Study participants will be followed up for cardiac and all-cause mortality outcomes until 1 year after the surgery."

D. Reviewer: 3
Dr. Damiano Regazzoli, IRCCS Humanitas Research Hospital
Comments to the Author:
While the study seems to be interesting, I have two major concerns:
1-It is not a contemporary study, at least for european countries

2-The results may be influenced by non-mitral surgery (I mean, that surgery that is not an exclusion criterium such as tricuspid or LAA procedures)
Furthermore, english grammar needs revisions.
I think that this protocol may be suitable for publication on a smaller or more specialistic Journal.
Authors' responses:
1. Thank you for your concerns and valuable suggestions.
RHD has been almost eradicated in European countries mainly attributed to improved living standards and widespread use of antibiotics. However, recent studies have shown that globalization, migration and refugee crises have led to increasing cases of RHD also in Europe and making RHD a global health problem. Ref I will send Reuben M et al.
Moreover for developing countries, rheumatic heart disease and especially its progression to rheumatic mitral stenosis is a great concern. Lack of studies focusing in the targeted therapies of rheumatic valve fibrosis in developing countries which need them the most, leads the authors to conduct this study, which hopefully could aid health centres in developing countries to face the morbidity of rheumatic heart disease, especially the rheumatic mitral valve disease.
2. The results may be influenced by non-mitral surgery procedures. We will make further multivariable analysis of these factors that may confound the results of this study. We state this concern in methods section, research techniques subsection, lines 180-182:
"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."
3. We have made major corrections for grammar and spellings. We used an English language editing service and natives to proofread our manuscript.

## VERSION 2 – REVIEW

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| **REVIEWER** | Gajbhiye, Rahul<br>NIRRH, Department of Clinical Research |
| **REVIEW RETURNED** | 09-Aug-2021 |

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| **GENERAL COMMENTS** | All comments are addressed in the revised manuscript. Best wishes for the implementation of the study. |

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| **REVIEWER** | Gupta, Prabha<br>Medical College, Cardiology |
| **REVIEW RETURNED** | 07-Aug-2021 |

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| **GENERAL COMMENTS** | As I stated earlier this is a very interesting study. However when I said about using the papillary muscles for staining I had one concern. Some surgeons do not resect the papillary muscles during surgery to preserve the architecture of the left ventricle .So if there are no papillary muscles available the study should be performed without them. |

## VERSION 2 – AUTHOR RESPONSE

Reviewer: 2
Dr. Prabha Gupta, Medical College
Comments to the Author:
As I stated earlier this is a very interesting study. However when I said about using the papillary

muscles for staining I had one concern. Some surgeons do not resect the papillary muscles during surgery to preserve the architecture of the left ventricle .So if there are no papillary muscles available the study should be performed without them.
Authors' answers:
Thank you for your valuable suggestions regarding the papillary muscle. We will attempt to get the papillary muscle too with the consideration of the cardiothoracic surgeons, case by case.
Reviewer: 1
Dr. Rahul Gajbhiye, NIRRH
Comments to the Author:
All comments are addressed in the revised manuscript. Best wishes for the implementation of the study.
Authors' answers:
Thank you for your valuable suggestions and support for this manuscript and hopefully, this study protocol will be implemented well.

**VERSION 3 – REVIEW**

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| **REVIEWER** | Gajbhiye, Rahul<br>NIRRH, Department of Clinical Research |
| **REVIEW RETURNED** | 25-Aug-2021 |

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| **GENERAL COMMENTS** | The manuscript is revised as per the comments |