Echocardiographic screening for subclinical rheumatic heart disease: Improving screening through simplification of the diagnostic criteria

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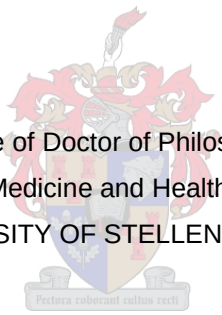

Dissertation Presented for the Degree of Doctor of Philosophy in the Department of Medicine
Faculty of Medicine and Health Sciences
UNIVERSITY OF STELLENBOSCH
December 2020

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Declaration

I, Luke David Hunter declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes three original papers published in peer reviewed journals and four unpublished, submission-ready manuscripts. The development and writing of the papers (published and unpublished) were the principal responsibility of myself and for each of the cases where this is not the case a declaration is included in the dissertation indication the nature and extent of the contributions of co-authors.

I am presenting this thesis for examination for the degree of PhD.

Dr LD Hunter

20th August 2020

Acknowledgements

My sincerest thanks and appreciation to the following organisation/ persons:

South African Heart Association: for the provision of a Doctoral research grant.

British Society of Echocardiography (BSE): to all the BSE members who gave of their time and skill each year during the Echo in Africa screening camps.

Colleagues and collaborators on all studies and papers presented in this thesis: Professor Mark Monaghan and Dr Guy Lloyd- who championed Echo in Africa from 'across the pond' and for their valuable input in each of the manuscripts presented in this thesis.

Dr Carl Lombard; for your ongoing support, guidance and statistical expertise throughout this journey

Kim Stanley: setting up Research Electronic Data Capture (REDCap) and continuous support through the first phases of the study.

Colleagues in the Division of Cardiology who supported me throughout this journey : Jane Moses, Bradley Griffiths, Lorita Kabwe, Rudolph du Toit, Lloyd Joubert, H.W Snyman, Jess John and Kariem Hassan.

Dr Alfonso Pecoraro: Flash Gordan en die 'man van staal': for your never-ending optimism, your echocardiographic and clinical mentorship and for your friendship and support over the last four years.

To all the study participants who gave freely of their time and their 'hearts'; your personal experiences of rheumatic heart disease and courageousness despite all odds, drove me to see this project to its end.

To all the doctors, sisters and clinic staff at each study enrolment site who welcomed me into their workplaces.

Professor Anton Doubell: my co-supervisor for this project. I am eternally grateful for your steadfast support of me throughout these four years of study. It has been an honour and privilege to be mentored by you.

Dr Philip Herbst: my supervisor and the person to whom I am indebted to for this rich experience. Thank you for joining me on this journey, for your wisdom, your considered opinion and for your example for which I hope to emulate.

To my dearest friends: the Lockyer and the Foster families for their ongoing support, love and fun.

To my parents, David and Celene Hunter and Sharon Faure: Thank you for all the ways which you have 'carried' me and my family over the last four years.

I dedicate this work to my dearest wife, Abby and our two children, James and Ella: for your unwavering support, patience and love, to my supervisors Dr Philip Herbst and Professor Anton Doubell and in loving memory of my dear friend Fritz Hörstmann.

Publications arising from this thesis

1. Hunter LD, Monaghan M, Lloyd G, Pecoraro AJK, Doubell AD, Herbst PG. Screening for rheumatic heart disease: is a paradigm shift required? Echo Res Pract. 2017 4(4) R43-R52 DOI: 10.1530/ERP-17-0037.

2. Hunter LD, Lombard CJ, Monaghan MJ, et al. Screening for rheumatic heart disease: The reliability of anterior mitral valve leaflet thickness measurement. *Echocardiography*. 2020;37(n/a):808-814. DOI:10.1111/echo.14751

3. Hunter LD, Monaghan M, Lloyd G, Pecoraro AJK, Doubell AF, Herbst PG. Prominent inter-scallop separations of the posterior leaflet of the mitral valve: an important cause of 'pathological' mitral regurgitation Echo Res Pract. 2018, DOI: 10.1530/ERP-18-0010

Abstracts presented at congresses with data from this thesis

1. Hunter LD, Doubell AF, Pecoraro AJK, Snyman HW, Lloyd G, Monaghan M, et al. Echocardiographic screening for rheumatic heart disease; the potential for misclassification of “borderline” cases. Eur Heart J 2018 Aug 1;39(suppl_1):ehy566.P5445-ehy566.P5445.
2. Hunter LD, Monaghan M, Lloyd G, Pecoraro A, Doubell A, Herbst P. Rheumatic heart disease in a “low risk” community : Are other risk factors at play. SA Heart 2018;15(4):283.
3. Hunter LD, Monaghan M, Lloyd G, Pecoraro A, Doubell A, Herbst P. Screening for rheumatic heart disease: A common normal variant of the posterior mitral valve leafl et resembles WHF-borderline rheumatic disease. SA Heart 2018; 15(4)284.
4. Hunter LD, Monaghan M, Lloyd G, Pecoraro A, Doubell A, Herbst P. The Echo in Africa project: A 5-year experience of cardiac screening in South African school children. SA Heart 2019; 16(3) 205
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Echocardiographic screening for subclinical rheumatic heart disease: Improving screening through simplification of the diagnostic criteria

Abstract

Rheumatic heart disease (RHD) remains one of the leading causes of cardiovascular morbidity and mortality in developing countries with Sub-Saharan Africa being identified as an endemic area. The early detection and initiation of secondary prophylaxis in children with 'latent' RHD remain attractive primary health care interventions, particularly in endemic regions with no or limited access to specialist cardiac services. However, the current consensus-derived screening criteria endorsed by the World Heart Federation (WHF criteria) are overly complex, require the use of expensive echocardiographic equipment with Doppler functionality and identify a large borderline diagnostic group that demonstrates a predominantly benign outcome in longitudinal study. This raises concerns regarding the feasibility of large-scale screening in resource-poor regions and questions the utility of early echocardiographic case-detection of RHD.

The primary purpose of this thesis was to critically appraise the performance of the WHF criteria and to determine whether a set of screening criteria based on a novel, focused morphological and mechanistic evaluation would simplify the current WHF guideline and reduce the number of cases 'misclassified' with borderline RHD whilst maintaining a similar degree of sensitivity.

A literature review was undertaken that critically appraised the performance of the current WHF criteria and its impact in African RHD screening programs. This highlighted important logistical and methodological shortcomings that have curtailed the implementation of large-scale RHD screening in RHD endemic regions. The five-year experience of a large-scale, high-risk RHD screening program (Echo in Africa [EIA] project) was analysed. The results from this project highlight RHD as an ongoing, significant healthcare challenge amongst underserved communities within the Western Cape, South Africa. The estimated prevalence of WHF 'definite-' and 'borderline-RHD' of 9.1 cases/1000 and 19.5 cases/1000 reported by EIA is significantly higher than that previously described in this region. Furthermore, a critical appraisal of the WHF criteria's performance in the EIA cohort highlighted various redundant and ambiguous criteria that require revision. Inter-scallop separations (ISS) of the posterior mitral valve leaflet (PMVL) were described in both our high- and very low-

risk populations. They were a common finding and the principal cause of WHF 'pathological' mitral regurgitation (MR) in the 'borderline RHD' group in both cohorts. This supported their status as a normal and importantly, non-rheumatic variant. The reliability of the current WHF anterior mitral valve leaflet (AMVL) thickness assessment was evaluated and was demonstrated to be poor amongst readers despite controlling for systematic bias. This raised the possibility of introducing a non-measurement-based AMVL thickness evaluation. A novel screening definition of AMVL restriction was introduced, enabling the description of a variable spectrum of AMVL restriction amongst children. This definition reliably identified two subtypes of leaflet restriction: a normal, 'gradual bowing' variant that localised predominantly to the medial portion of the leaflet and a 'distal tip' variant seen to affect at least the central portion of the leaflet in all cases of WHF 'definite RHD' in this cohort. Finally, this thesis culminated in the development and evaluation of a novel set of morpho-mechanistic (MM) echocardiographic screening criteria for RHD. Together with an abbreviated 'rule-out' screening test, the MM criteria were assessed alongside the current WHF criteria in a gold standard RHD-negative cohort and a gold standard RHD-positive cohort. The MM criteria significantly reduced the false-positive rate of a borderline diagnosis in the gold standard RHD-negative cohort (2.7/1000 vs 41.8/1000) whilst maintaining a similar screening sensitivity (99.7%) compared to the WHF criteria (95.9%) within the gold standard RHD-positive cohort. Similarly, the MM RHD 'rule-out' test performed well by excluding the majority of cases (98%) within the gold standard RHD-negative cohort while including all cases within the gold standard RHD-positive cohort.

The work presented in this thesis addresses key research needs and gaps in our current understanding of 'screen-detected' latent RHD. It represents a significant contribution that will impact on policy, practice and further research in the field. The discovery that ISS of the PMVL are a normal finding and the principal cause of isolated 'pathological' MR in the borderline group represents a key element in solving the 'borderline conundrum'. This discovery supported the adoption of a morpho-mechanistic screening approach over a predominantly functional MV assessment. Centred around a novel definition of AMVL restriction, the MM criteria significantly improve the specificity of RHD detection by markedly reducing the size of the borderline group. Importantly, this was achieved without a reduction in the sensitivity of the criteria when compared to the current WHF criteria. Together with a simple 'rule-out' test, the MM criteria bring us closer to the objective of implementing large-scale screening programs that identify children with latent RHD who will benefit from secondary prophylaxis.

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Introduction and review of the literature

Rheumatic heart disease (RHD) remains one of the leading causes of cardiovascular morbidity and mortality in developing countries, with an estimated 15.6 million people affected worldwide.[1] The annual global incidence of RHD is estimated at 200 000-300 000 cases with a similar number of deaths that are attributed to the disease.[1] Sub-Saharan Africa has been identified as an endemic area with an estimated one million children living with RHD, amounting to almost half of the affected children in the developing world.[1] It is hypothesised that the global burden of RHD may have been underestimated due to limitations in the studies incorporated in analyses to extrapolate prevalence data.[2] Despite being identified as an endemic region, the prevalence of RHD in South Africa is largely unknown due to a lack of published data from echocardiographic screening programs.[3,4] Rheumatic heart disease is thought to be a sequela of a delayed autoimmune reaction to group A streptococcal infection.[5] An estimated 0.3-3% of those with untreated group A beta-haemolytic streptococcal infection progress to develop acute rheumatic fever (ARF) and approximately 40-60% of episodes of ARF are associated with carditis that progress to RHD.[6] The disease process is initiated in childhood and is prevalent in populations with low income, crowded living conditions and poor access to quality healthcare.[6] The medical complications associated with RHD include heart failure, infective endocarditis, atrial fibrillation, pregnancy-related complications and stroke. [1,2,7] This underlines the importance of screening children in high-risk populations.

The hallmark feature of RHD is chronic valvular damage thought to result from episodes of acute valvulitis. This is characterised histologically by small vegetations (verruca) on the leaflets, active inflammation and oedema along with focal evidence of infiltration of immune cells and neoangiogenesis (Aschoff nodules).[8] The process culminates in fibrosis of the valve and can render it incompetent and/or stenotic. As both the index infection and the recurring bouts of streptococcal pharyngitis are susceptible to penicillin therapy, RHD remains a potentially preventable condition and thus should be the focus of primary health care initiatives that promote the early identification and treatment of suspected bacterial throat infections.[9]

The role of screening echocardiography and the WHF criteria

To date, there is no unequivocal diagnostic test that identifies a person as having RHD. Echocardiography has been identified as being the diagnostic investigation of choice, far outperforming previously adopted auscultation based screening protocols and has recognised a large subgroup of patients with previously undetectable disease (subclinical RHD).[10,11] Due to the systematic differences in the reporting of and the diagnostic approach to subclinical RHD, the World Heart Federation (WHF) developed a set of consensus-based criteria– the 2012 WHF criteria for echocardiographic diagnosis of RHD.[12] The screening criteria incorporate data obtained from two dimensional (2D), continuous wave (CW) and colour Doppler measurements and are designated for use in individuals aged 20 and younger with no previous history of ARF. The screened case in individuals ≤20 years of age is ascribed to one of three categories: a WHF ‘definite RHD’ group (requiring both a typical morphological and functional abnormality which is identified as ‘pathological’ by meeting all four Doppler criteria), a WHF ‘borderline RHD’ group (either, a typical

morphological rheumatic feature or a 'pathological' functional abnormality) and a 'normal group' (non-diagnostic morphological abnormality or regurgitation not meeting all four Doppler criteria).

Limitations of the current WHF criteria screening methodology

The criteria have been widely adopted and have indeed resulted in a wealth of standardised data generated from numerous large scale screening programs around the globe.[3,13–16] However, various factors have been identified as potential pitfalls to the effective implementation of the WHF criteria in middle- and low-income countries. These include:

1. **The overall complexity of the current screening guideline that limits its application by non-experts in-the-field**.[17–19]

The WHF criteria are complex and require that a screener with a high level of expertise is utilised to effectively screen cases. These trained health care workers are limited in countries where access to specialist care remains restricted.[19]

2. **The inclusion of a Doppler-based regurgitation severity assessment that offers little to no information regarding the underlying aetiology of dysfunction**.[20]

In clinical practice, a morphological and mechanistic assessment is typically used to identify the cause of valvular regurgitation as the severity of a functional deficit contains very little if any information of aetiology by itself. The WHF guideline acknowledges the notion that isolated 'pathological' MR/AR could well incorrectly designate a case as 'borderline RHD' and emphasised the need to "*exclude congenital, acquired and degenerative heart disease of the MV and AV before presuming rheumatic origin*." [20] This crucial step was seemingly omitted in several recently published RHD screening studies[10,17,21] as "*congenital valvular anomalies were not recognised and could well have led to an overestimation of the RHD prevalence*".[22]

3. **The requirement for echocardiographic devices that have Doppler functionality.**

The 'standalone' and portable laptop echocardiography machines are both expensive and dependent on a supply of electricity, making them unattractive options for use in a resource-limited setting including screening in-the-field. Handheld echocardiographic devices herald an attractive solution for large-scale screening programs as they are portable and battery-powered. However, these devices do not offer a satisfactory 'point-of-care' measurement function or Doppler functionality required to apply the full WHF criterion.

4. **The size of the borderline diagnostic category**.

Subclinical RHD incorporates a spectrum of echocardiographic findings ranging from non-specific changes to features pathognomonic for RHD. The WHF 'definite RHD' criteria perform well in the identification of cases with true RHD. However, the 'borderline RHD' diagnostic category introduced to improve the sensitivity of the guideline has resulted in the identification of a large, diverse indeterminate group of cases with unknown clinical significance. As a result, the WHF guideline does not advocate that patients with 'borderline RHD' disease receive penicillin prophylaxis. This has become the subject of much debate amongst members of the

RHD research community,[14,19,23–26]with the suggestion that the use of screening echocardiography in subclinical RHD should, for now, be viewed as a research tool, pending more definitive studies on the prognosis of 'screen-positive' cases.[27]

Despite the current controversies surrounding the WHF 'borderline RHD' group, there is little doubt that the current criteria identify a proportion of cases with true RHD in the borderline group. This is supported by an Australian cohort study demonstrating that some children diagnosed with 'borderline RHD' are at an increased risk of ARF, the progression of cardiac valvular lesions and the development of WHF 'definite RHD'. [25]

Consequently, there remain critical research priorities in the field of echocardiography and RHD screening. The first is to determine whether the current WHF screening criteria can be sufficiently simplified and revised to enable its efficient use by non-experts in-the-field with handheld devices. Second, is the need to address the WHF's suboptimal screening specificity by determining novel, alternate echocardiographic features that either increase or decrease the likelihood of an underlying true diagnosis RHD.[14]

Specific research objectives of this thesis

The objectives of this thesis are to:

1. Analyse data from the first five years of the Echo in Africa (EIA) project -a large-scale echocardiographic screening program in the Western Cape.

2. Critically appraise the performance of the WHF criteria and determine key elements that require revision that would simplify a screening algorithm.

3. Investigate morphological and mechanistic features that could better define the presence or absence of true RHD.

4. Validate a new set of echocardiographic criteria for the echocardiographic diagnosis of RHD.

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Chapter 1: Screening for rheumatic heart disease: is a paradigm shift required?

Chapter one is a published focused review presenting a critical appraisal of the World Heart Federation (WHF) criteria for the echocardiographic diagnosis of rheumatic heart disease (RHD) and its performance in RHD screening programmes in Africa. I am the principal author of this article.
MJ Monaghan, GW Lloyd and AJK Pecoraro reviewed the final draft of the manuscript. AF Doubell and PG Herbst were the co-supervisor and supervisor respectively. Both reviewed the final draft of the manuscript.

Published manuscript

Screening for rheumatic heart disease: is a paradigm shift required?
LD Hunter, MJ Monaghan, GW Lloyd, AJK Pecoraro, AF Doubell, PG Herbst
Echo Research and Practise. 2017 Dec;4(4):R43–R52. doi:10.1530/ERP-17-0037

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1.1. Background

Rheumatic heart disease (RHD) remains one of the leading causes of cardiovascular morbidity and mortality in developing countries.[1] Sub-Saharan Africa has been identified as an endemic RHD region with extrapolated figures estimating the disease burden of latent RHD to be anywhere from 1.1 to 13.2 million.[2] To address the burden of RHD on the continent, the African Union adopted the Addis Ababa communique[3] at the 25th African Union Heads of State and Government Summit held in Johannesburg, 2015. The communique is a seminal

position statement devised by RHD clinicians and researchers affiliated with the Pan-African Society of Cardiology (PASCAR) and outlies seven priority areas of action for the eradication of RHD in Africa. The fourth recommendation of the communique recognises the pivotal role that cardiac ultrasound will fulfil to assist in "*the early detection, diagnosis, secondary prevention and treatment of RHD".*[3] However, an incomplete understanding of the natural history of latent RHD, coupled with various deficiencies in the current RHD echocardiographic diagnostic guideline have precluded its endorsement for use in large scale echocardiographic screening programs.

This article will review the 2012 World Heart Federation (WHF) echocardiographic criteria for the diagnosis of RHD and its performance in African RHD screening programs. It hopes to outline the various deficiencies inherent to the current guideline and highlight novel alternative methods of echocardiographic RHD identification that may improve the performance of screening criteria.

1.2. The role of echocardiography in RHD screening

The efficacy of secondary prevention in acute rheumatic fever (ARF) is well documented and originates from current understanding that individuals with a previous history of ARF are predisposed to recurrent attacks which can be prevented by the administration of regular prophylactic antibiotics.[4-6] However, the accurate identification of those with an increased risk is fraught with complexities as it is estimated that up to 40% of individuals with established RHD have no recollection of having symptoms compatible with an ARF episode.[7] This provides an ideal opportunity for disease control programs to institute targeted screening to identify those individuals at risk for further progression to symptomatic disease. Prior to the advent of echocardiography and its utility in RHD diagnosis, RHD screening programs relied on cardiac auscultation to identify potential cases of RHD. Most of the published prevalence rates of antecedent RHD screening programs in Africa ranged from 1.0 to 10.2/1000.[8-12] However, echocardiography has since proven to be a sensitive screening tool with detection rates of RHD considerably higher than that of its auscultation-based counterpart with prevalence rates in Africa as high as 30.4/1000.[13] The prospect of early detection of subclinical disease (asymptomatic individuals with no previous history of ARF) coupled with the presumed efficacy of secondary prophylaxis to avert progression to severe symptomatic disease led to a reinvigoration of African RHD research. [14-20]

1.3. The 2012 World Heart Federation Criteria

Due to the systematic differences in the diagnostic approach and reporting of screening echocardiograms in subclinical RHD, the World Heart Federation (WHF) developed a set of consensus based criteria– the 2012 WHF criteria for echocardiographic diagnosis of RHD.[21] (Table 1.1)

The criteria have been widely adopted and have resulted in the publication of a wealth of standardized data that document a latent RHD disease burden of epidemic proportions amongst African school-going children.[22-32] This has provided an impetus for African countries to endorse the recommendations of the Addis Ababa communique and amend health policy accordingly to include routine RHD screening. However, the screening experience whilst utilising the WHF criteria has also raised sufficient concern to limit its implementation in

resource-restricted areas.[33-35] This is due to various methodological and performance-related issues that will require further scrutiny and possible amendment should large-scale RHD screening be endorsed in the future.

These concerns are broadly summarised and discussed as follows:

1.3.1. The state of African healthcare systems
1.3.2. The logistical requirements of the WHF criteria
1.3.3. Simplification of the WHF criteria
1.3.4. Methodological deficiencies in the WHF criteria
1.3.5. The natural history of subclinical RHD

1.3.1. The state of African healthcare systems

The Addis Ababa Communique identifies the importance of decentralising the diagnostic services for RHD to district and primary healthcare hospitals in Africa. This involves the training of designated healthcare workers in echocardiography and the provision of adequate ultrasound equipment, technical support and basic infrastructural requirements to create a sustainable service. However, this poses a massive challenge to African countries whose overextended health systems are limited by budgetary constraints, excessive disease burden and dire shortages of skilled staff.[36] Furthermore, an important limitation that has been described in African RHD literature is the frequency of enrolled participants who are subsequently 'lost to follow-up'. This is attributed to various factors which include a high 'drop-out rate' amongst school-children, a "*migratory culture*" amongst certain communities and poor access to mobile phone technology. [23,24,37] Although these difficulties are inherent in any study, they are nonetheless obstacles that can impact significantly on the success of a program. The minutiae detailing present healthcare constraints and the reform that is required to successfully implement effective RHD screening in African countries lie outside the scope of this article. These challenges however must be borne in mind as they represent possibly the most significant obstacle to the institution of a successful screening program in resource-poor settings.

1.3.2. The logistical requirements of the WHF criteria

To provide an evidence-based guideline for the detection of RHD, a screened case with either mitral or aortic valve regurgitation is evaluated according to specific Doppler-based measurements (Table 1. 1). These include various spectral Doppler parameters that effectively limit the 'gold standard' technology with which to effectively screen for RHD to echocardiographic machines that are equipped with this functionality. These units are expensive and are dependent on a reliable supply of wired electricity making them unattractive options for use in a resource-limited setting.[30,32]

The advent of the handheld echocardiographic device has heralded an attractive solution for large scale screening programs as they are portable, battery powered and marketed at a fraction of the cost of the conventional machines. The advantages of portability and cost of the units are however somewhat offset by various technological issues that require further elucidation.

Firstly, the most notable disadvantage of the current handheld devices is the absence of spectral Doppler functionality, which as previously indicated is mandatory for the successful utilisation of the current criteria. Secondly, the unit scans with obligatory Tissue Harmonic Imaging (THI) that could explain the observation made by Beaton *et. al* [30] of thicker cardiac structures and increased false-positive diagnoses of chordal thickening and leaflet restriction in their studied cohort. In addition, the WHF guideline recommends that anterior mitral valve leaflet thickness measurements obtained using THI should be cautiously interpreted and a thickness of up to 4mm should be considered normal in individuals ≤20 years of age.[21]Thirdly, the potential discrepancies in the leaflet assessment are further exacerbated by a basic 'point-of-care' measurement tool that is limited to one millimetre increments and has been recognised to overestimate leaflet thickness.[30] Lastly, the units require regular recharging due to a limited battery lifespan and overheat during prolonged scanning with the added risk of a reduction in scanning frame rate. [31,32,38]

1.3.3. Simplification of the WHF criteria

Recent RHD research has focussed on simplifying the current criteria to enable its incorporation into handheld screening protocols.[25,29-31,39-40] The use of a single mitral regurgitation (MR) jet-length measurement to denote RHD is an attractive option, but may contrive to cause undesirable consequences.

Firstly, validation of the 'focused' protocol becomes problematic as the same parameter remains at the crux of the comprehensive WHF functional assessment and risks confirmation bias.[42] Secondly, it risks missing true rheumatic disease cases with either isolated morphological features or a functional assessment measurement just below the cut-off value (reducing sensitivity of the criteria)[7]. Thirdly, an additional case-load of alternative causes of 'pathological' MR could be included in this subset (reducing specificity), which may overburden the tertiary referral- care services and swamp the *"already stretched paediatric cardiology services"* [7] Fourthly, it overlooks the finding of Marijon *et al.* who noted that their 'combined criteria' (requiring features of chronic morphological RHD and any degree of regurgitation) led to a markedly improved detection rate of RHD as compared to a functional Doppler assessment alone.[43] Lastly, the impact of a false-positive result on an individual patient-level cannot be discounted and would undoubtedly result in unnecessary anxiety and the inappropriate prescription of long-term secondary prophylaxis.[7,44]

1.3.4. Methodological deficiencies in the WHF criteria

Lack of a RHD-specific scanning protocol

A challenging aspect of RHD screening remains the identification of subtle structural changes that are recognised to only affect specific leaflet segments. The WHF guideline recognises this and cautions that some children with pathology will be missed if only "*standard, adult-style echocardiographic views are assessed".* [21]

The current guideline however, does not define a standardised screening protocol that will successfully identify subtle RHD pathology. The validation and subsequent introduction of a tailored screening protocol for

RHD identification could improve the overall standard of screening and potentially reduce the amount of missed RHD cases.

The Doppler criteria and alternative causes of 'pathological' MR

The Doppler criteria stem from early Doppler-work that identified its potential to effectively differentiate between physiological and 'pathological' regurgitant jets.[45-48] This body of research was incorporated into echocardiographic criteria used to identify subclinical ARF carditis [49,50] and later RHD.[51]The Doppler criteria were amalgamated into the current 2012 WHF criteria largely based on data suggesting that 'pathological' MR was more likely to be observed in children in high-risk RHD areas than low risk RHD areas [52] (Table 1. 1).

The criteria however have been identified as a shortcoming of the current WHF guideline for two principle reasons. Firstly, they comprise a set of somewhat arbitrary and redundant parameters which include a non-physiological regurgitant jet velocity cut-off [42,53] , a requirement to identify the jet in two views (testing only the screener's ability)[42], the requirement of a pan-systolic/pan-diastolic jet which provides no additional information regarding the mechanism of regurgitation[42] and a jet length measurement that is subject to interobserver variability and whose specificity in identifying disease progression has been questioned.[26] Use of the current Doppler criteria could risk labelling screened cases of arguably true RHD (with specific morphological features of RHD) as WHF 'borderline RHD' because they are deficient in any one of the measured Doppler parameters (Figure 1. 1 and Figure 1. 2 and for corresponding media clips refer to Media clip 1. 1and Media clip 1. 2).

Secondly, the incorporation of a 'borderline RHD' category to improve the sensitivity of the WHF criteria has illuminated the Doppler criteria's lack of specificity. This is exemplified by the finding of 'pathological' MR that was attributable to congenital mitral valve(MV) variants in screened cases from both high- and low-risk populations [24,52-55] (Figure 1. 3, Figure 1. 5, Figure 1. 6 and for corresponding media clips refer to Media clip 1. 3, Media clip 1. 4, Media clip 1. 5, Media clip 1. 6).

The WHF guideline made provision for this contingency by adding a pre-requisite that "*congenital, acquired and degenerative heart disease of the MV and AV"* are excluded before presuming rheumatic origin.[21] The guideline further adds that "*congenital cardiac defects are easily differentiated from RHD, as they have unique identifying features (for example, bicuspid AV or MV cleft)."* [21] Whilst this may be true for entities such as the bicuspid AV, MV cleft and MV prolapse that have been well described in both anatomical pathology and echocardiographic literature and have pathognomonic echocardiographic features that identify them as such. The premise however does not hold true for all cases that are identified as WHF 'borderline RHD' based on an isolated 'pathological' MR jet. A subset of these cases has been alluded to in current RHD literature as being on the *"upper limit of physiological mitral valve regurgitation"* [56] or screened cases with *"minor congenital MV anomalies".* [53] However, the exact mechanism of valvular incompetence in these cases has not been identified.

An additional cause for concern is the description of an entity identified in South African high risk children that may be mistakenly identified as potential RHD. These have been described as normal spectrum mitral valves

with WHF-'pathological' regurgitation identified through "*prominent posterior leaflet inter-scallop separations*."[42] Currently it remains unclear as to whether these "*inter-scallop separations*" are related to similar entities described in the literature as posterior mitral valves with "*isolated clefts*"[57], "*subclefts*[58]","*interscallop malcoaptations*"[57] and "*slits*"[59]. It is evident that more work is required to investigate and describe the aetiology, common echocardiographic characteristics and clinical course of non- rheumatic mitral valves which display WHF 'pathological' MR.

1.3.5. The natural history of subclinical RHD

An early echocardiographic diagnosis of subclinical RHD has particular bearing for screened cases in resource-poor African countries. In these communities, the management options for individuals with symptomatic severe RHD become extremely limited due to constrained cardiothoracic/interventional cardiology services.[60] Individuals identified with subclinical disease in these instances would intuitively benefit the most from the early institution of an appropriate secondary prophylaxis regimen to avert progression to symptomatic disease.

However, the efficacy of secondary prophylaxis to prevent further ARF recurrences and progression of clinically detectable RHD cannot be automatically extrapolated to include screened cases with subclinical RHD.[56] This is in part related to the paucity of long term echocardiographic follow-up studies utilising standardised diagnostic and reporting methodology.[21] Furthermore, the establishment of a randomised control trial (RCT) evaluating prophylaxis versus no prophylaxis in subclinical RHD is controversial as it is considered that withholding prophylaxis to an individual with WHF-identified 'definite RHD' is unethical.[56]

The diagnostic confidence that a WHF 'borderline RHD' diagnosis conveys however is not as robust. The borderline group was introduced to improve the sensitivity of the guideline at the expense of the specificity and has resulted in the identification of a large, diverse indeterminate group of cases with unknown clinical significance. Accordingly, the WHF guideline does not advocate that patients with WHF 'borderline RHD' disease receive penicillin prophylaxis. This has become the subject of much debate amongst members of the RHD research community with the suggestion that the use of screening echocardiography in subclinical RHD should for now, be viewed as a research tool, pending more definite studies of impact on prognosis. [7,33,52-55,61,62]

Five research groups who have followed cohorts of screened WHF subclinical RHD cases have subsequently published their findings [26,33,37,62,63](Figure 1. 7). Despite various limitations which include small cohorts and relatively short term follow-up, the studies do provide a preliminary insight into the natural history of WHF subclinical disease and may highlight important principles that are deficient in the current guideline.

All five publications identify that the natural history of WHF 'borderline RHD' is not necessarily benign (Figure 1. 7). There is a variable, yet significant proportion of borderline cases that have been demonstrated to persist at follow–up and a smaller population displaying progression to WHF 'definite RHD'. Despite the documented risk of disease persistence and progression, the hallmark of WHF 'borderline RHD' was its predilection to

revert back to normal with so-called "*disease regression*" demonstrated in the majority of these longitudinal studies (Figure 1. 7). Various reasons have been offered to account for these findings that include issues with inter-observer variability [37,63] , the administration of secondary prophylaxis [26], the inability of the WHF criteria to classify screened individuals >20 years of age into a borderline group[37], or even that subclinical RHD represents a disease process that can resolve back to normal in a large majority of cases.[37]

The notion of disease regression and improvement of 'pathological' lesions whether they be morphological or functional raises some important issues that beg further investigation. All else being equal, one would expect that chronic RHD morphological abnormalities such as thickening and restriction of the valvular and subvalvular apparatus will persist and are unlikely to improve over time. The identification of these morphological features could therefore represent the most specific predictor for true RHD.[26,62,63]

If this hypothesis is demonstrated to be true, could the finding of subclinical RHD disease regression be a false representation of the natural history of true RHD and could the current WHF screening methodology be responsible for perpetuating this anomaly?

1.4. Alternative RHD screening methodologies

A recent commentary of the WHF criteria[42] has proposed an alternative RHD screening methodology that deviates from the precepts incorporated in the current guideline.
The commentary argues that the pattern of "*diastolic leaflet restriction*" remains a principle finding in RHD and advocates that a comprehensive leaflet assessment be assimilated into a screening protocol to identify subtle focal RHD involvement. It further recognizes that the current morphological and functional assessment comprise inherent technical and methodological pitfalls that necessitate further scrutiny and potential amendment as they may impede on the guideline's performance. The most notable amendment proposed in this piece is that the presence of regurgitation (of any degree) in a screened valve should prompt an active search for the mechanism of dysfunction. This so-called 'mechanistic evaluation' would be incorporated in lieu of the current Doppler assessment and could potentially discriminate between subtle cases of true RHD and the extraneous mimics of RHD identified in the WHF 'borderline RHD' category. This approach, although untested in RHD screening may prove to be of merit as it echoes the general principles expounded in current echocardiographic recommendations for the evaluation of native valvular regurgitation.[64]

1.5. Conclusion

The establishment of the World Heart Federation criteria for the echocardiographic diagnosis of RHD represents a significant endeavour to combat the scourge of RHD across the globe. The guideline has undoubtedly standardised the process of disease identification, kindled further RHD research ventures across the African continent and deepened our understanding of subclinical disease progression. Above all, the criteria have highlighted the excessive burden of disease across the continent and with it prompted African leaders to implement large scale health policy reform. However various logistical and methodological shortcomings have prevented its endorsement in large scale screening programs and cast doubt on the

findings of long term cohort studies of subclinical disease. At the heart of some of these shortcomings lies the difficulty of accurate RHD case detection using echocardiography. Our pursuit to improve this accuracy may necessitate a paradigm shift in the echocardiographic approach we use.

1.6. References

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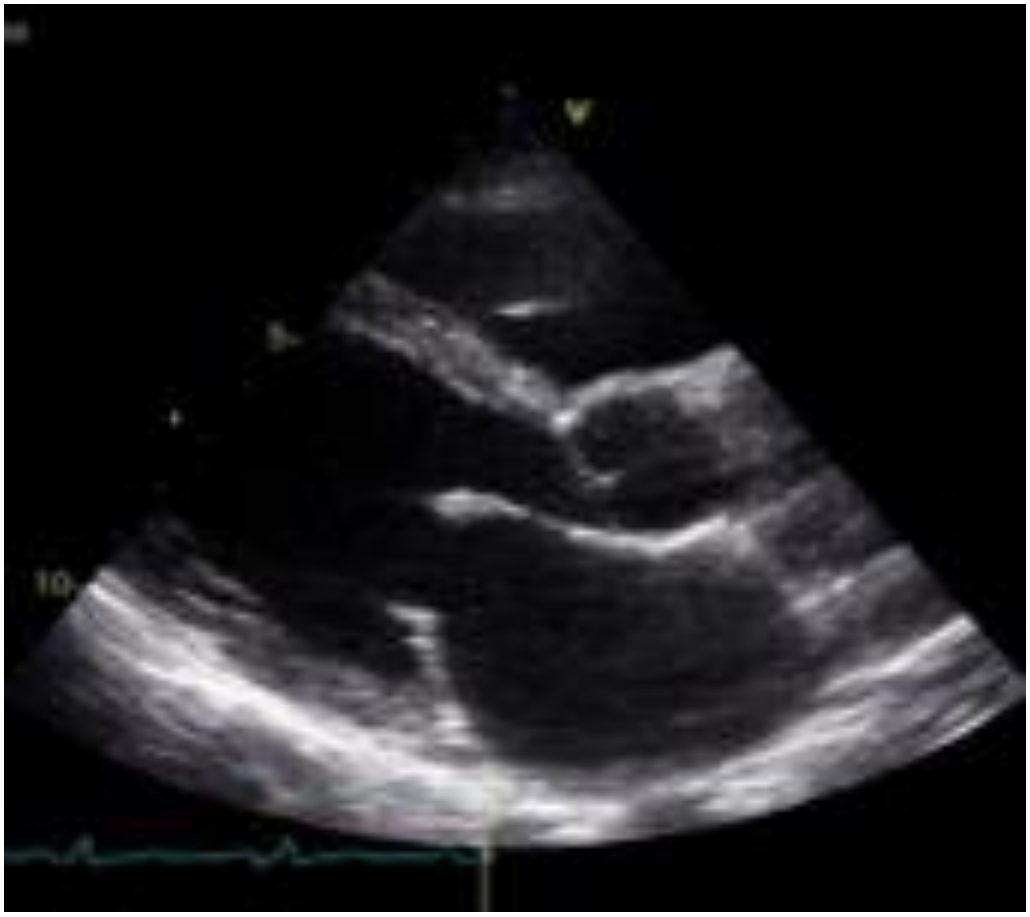
1.7. Figures

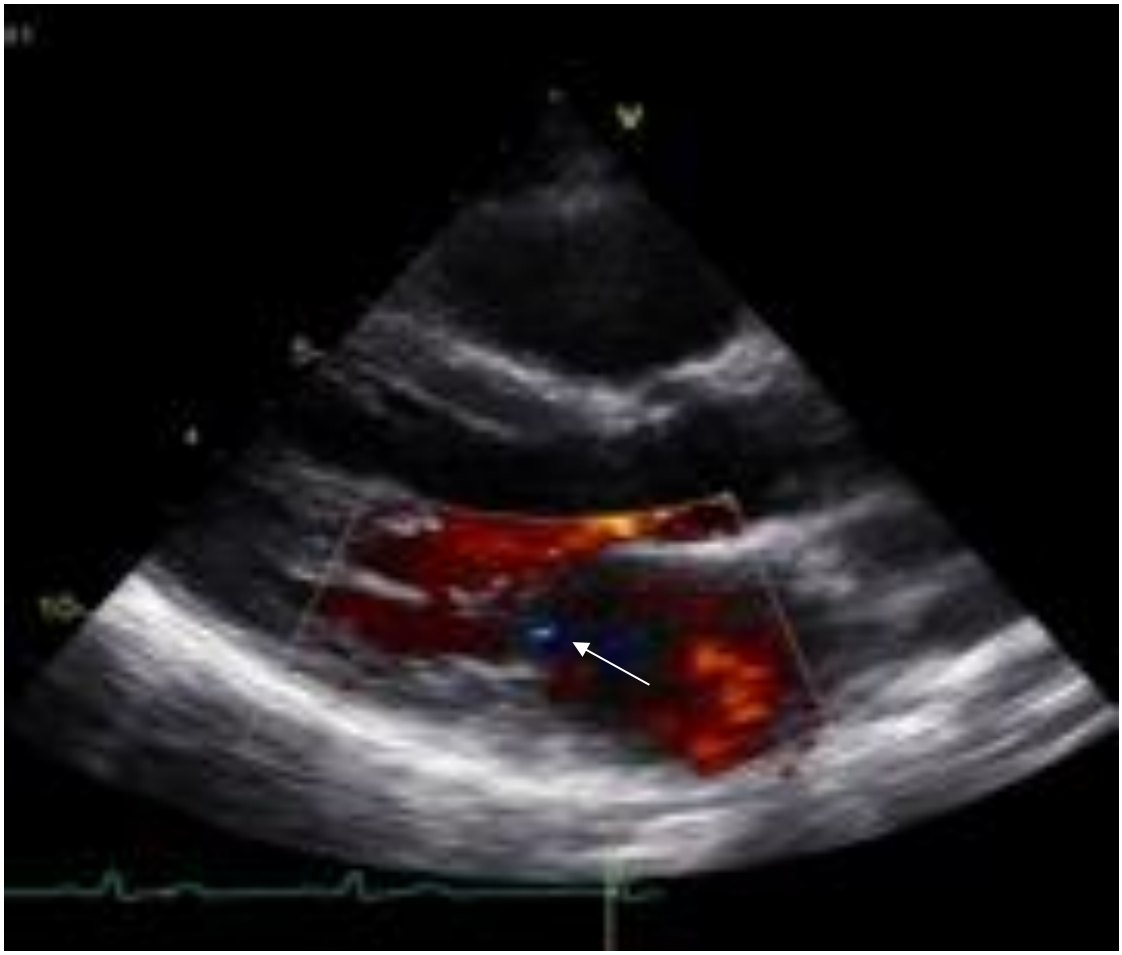
Figure 1. 1. Still image of a mitral valve with typical RHD morphological features



Still image taken from a screening 2D echocardiogram in a parasternal long-axis view. There are morphological features of RHD of the mitral valve (diastolic restriction of both leaflets with thickening of the leaflet tips).To view the corresponding media clip, refer to Media clip 1.1.

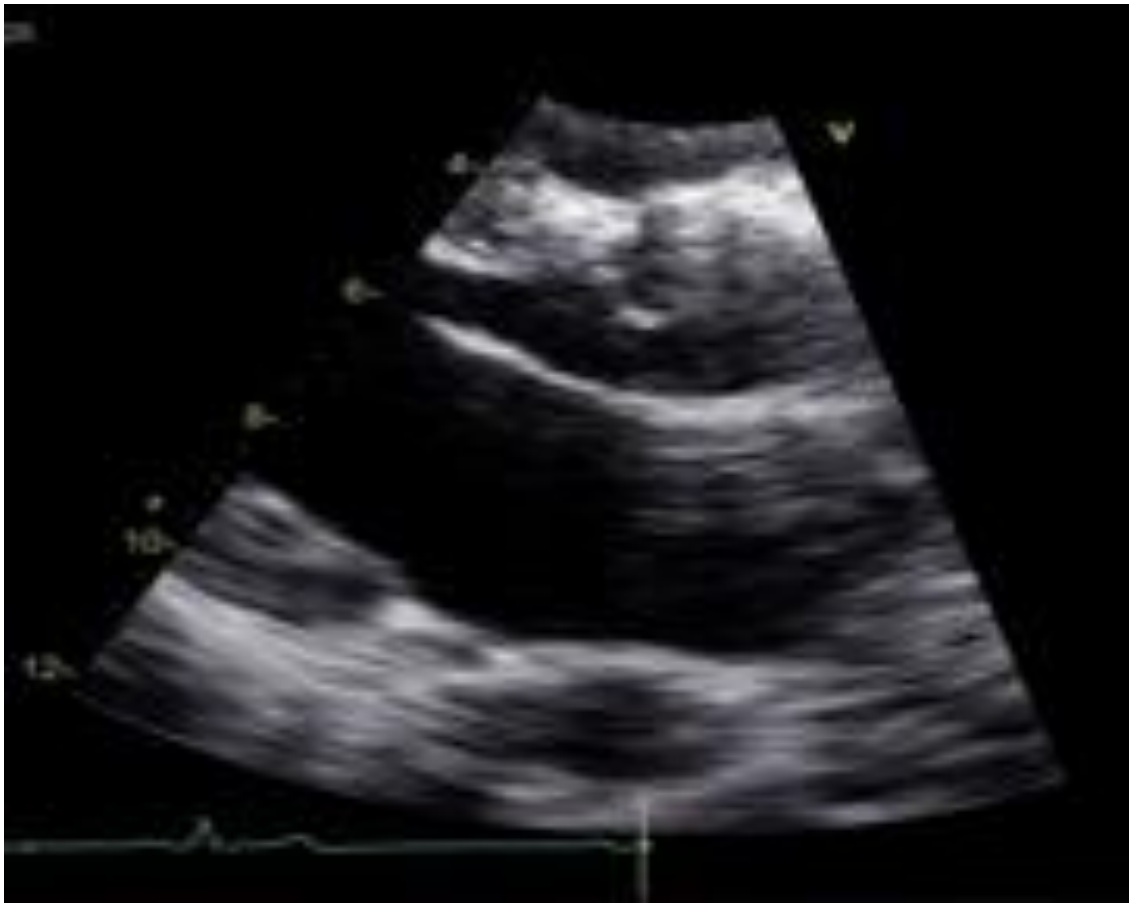
2D, two-dimensional; RHD, rheumatic heart disease

Figure 1. 2. Still image of a mitral valve with typical RHD morphological features with focused colour over the mitral valve.



Still image of corresponding case in Figure 1.1 with focused colour Doppler over the mitral valve(MV). The white arrow shows pixel mitral regurgitation during ventricular systole. The regurgitant jet measured <2cm and therefore the case was designated as WHF ‘borderline RHD’. To view the corresponding media clip, refer to Media clip 1.2.

Figure 1. 3. Still image of a normal mitral valve

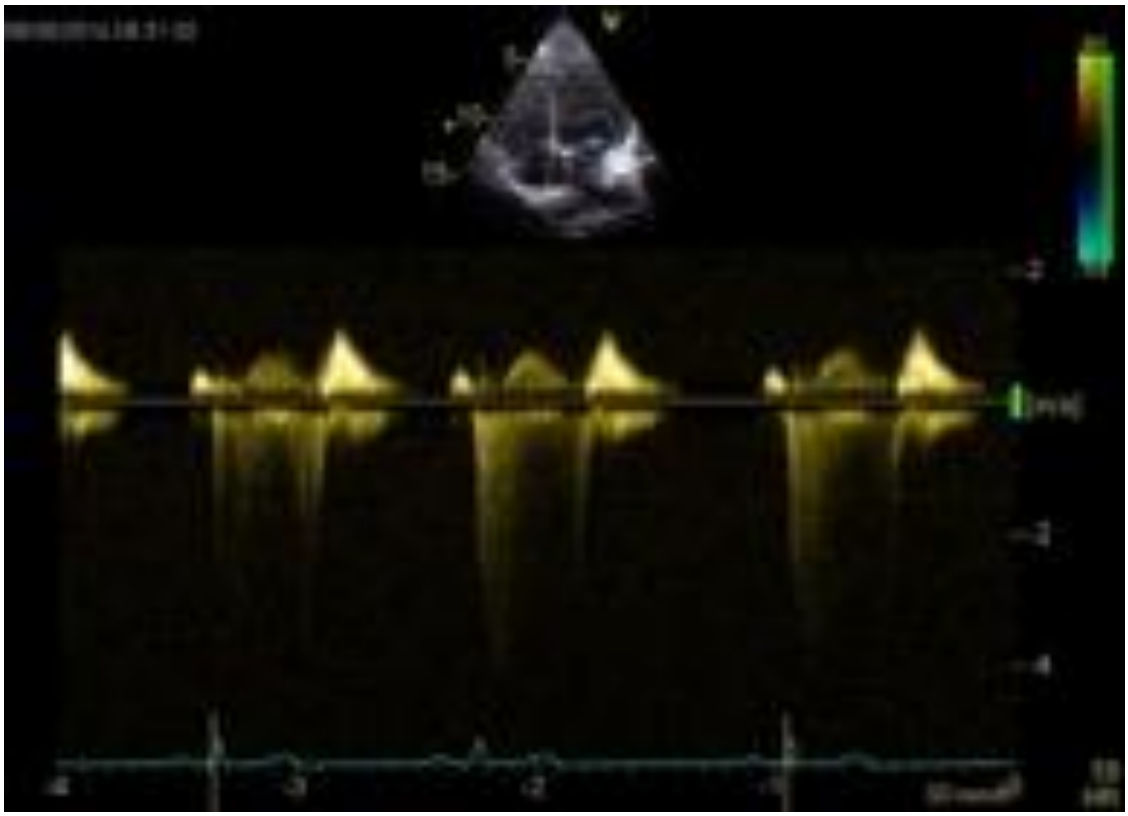


Still image taken from a screening 2D echocardiogram in a parasternal long axis view with MV leaflets at maximal diastolic excursion. There are no morphological features of RHD of the MV
(both leaflets are thin and demonstrate no diastolic restriction). To view the corresponding media clip, refer to Media clip 1.3.

Figure 1. 4. Still image of corresponding case in Figure 1.3 with focused colour Doppler over the mitral valve.

Still image of corresponding case in Figure 1.3 during ventricular systole with focused colour Doppler over the MV. The white arrow shows WHF ‘pathological’ mitral regurgitation during ventricular systole. The regurgitant jet measured >2 cm and met all additional Doppler criteria. The screened case is therefore case designated ‘borderline RHD’. To view the corresponding media clip refer to Media clip 1.4.

Figure 1. 5. Continuous wave trace through the mitral valve of the corresponding case in Figure 1.3.

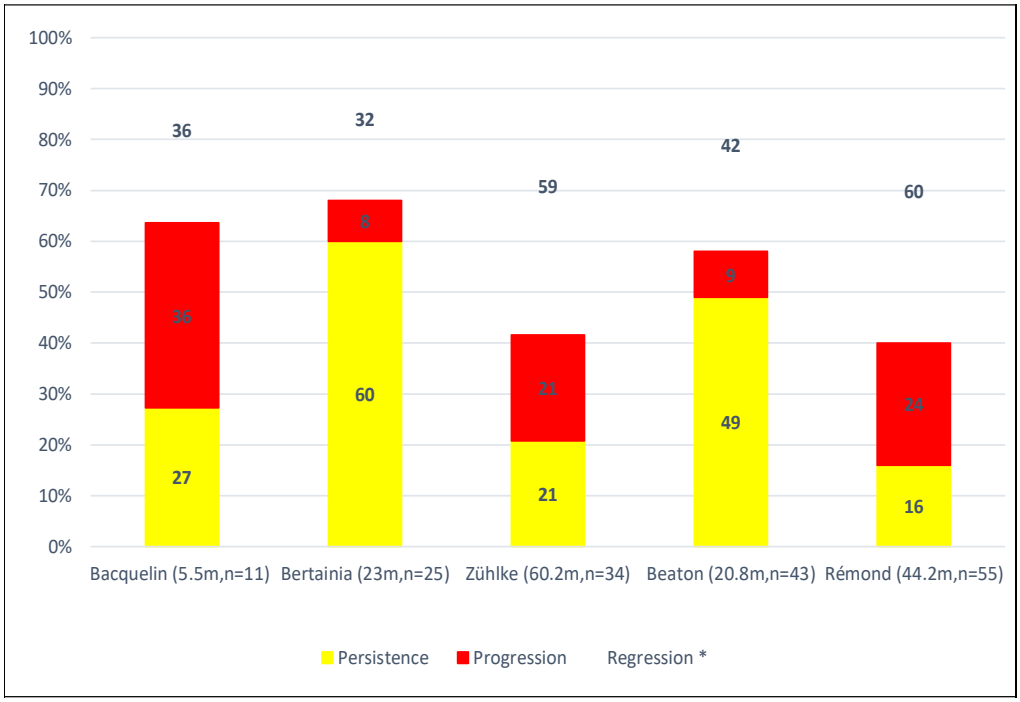


The continuous-wave Doppler trace confirms a pansystolic jet with a complete envelope and a peak velocity > 3m/s.

Figure 1. 6. Still image of corresponding case in Figure 1.3 in parasternal short-axis view demonstrating an inter-scallop separation of the posterior mitral valve leaflet.

The white arrow shows a prominent inter-scallop separation of the posterior leaflet. Focused colour Doppler over the MV subsequently demonstrated the inter-scallop separation to be the cause of the incompetence. To view the corresponding media clip refer to Media clip 1.5.

Figure 1. 7. A comparison of the natural history of borderline rheumatic heart disease in five screening studies.



A comparison of the natural history of borderline rheumatic heart disease in five studies with increasing number of studied participants (m, mean duration of follow-up in months; n, sample size of borderline cases). *Rémond and coworkers's publication only presented persistence and progression data from their cohort. The presented regression data are thus inferred considering the total number of borderline cases that were followed up.

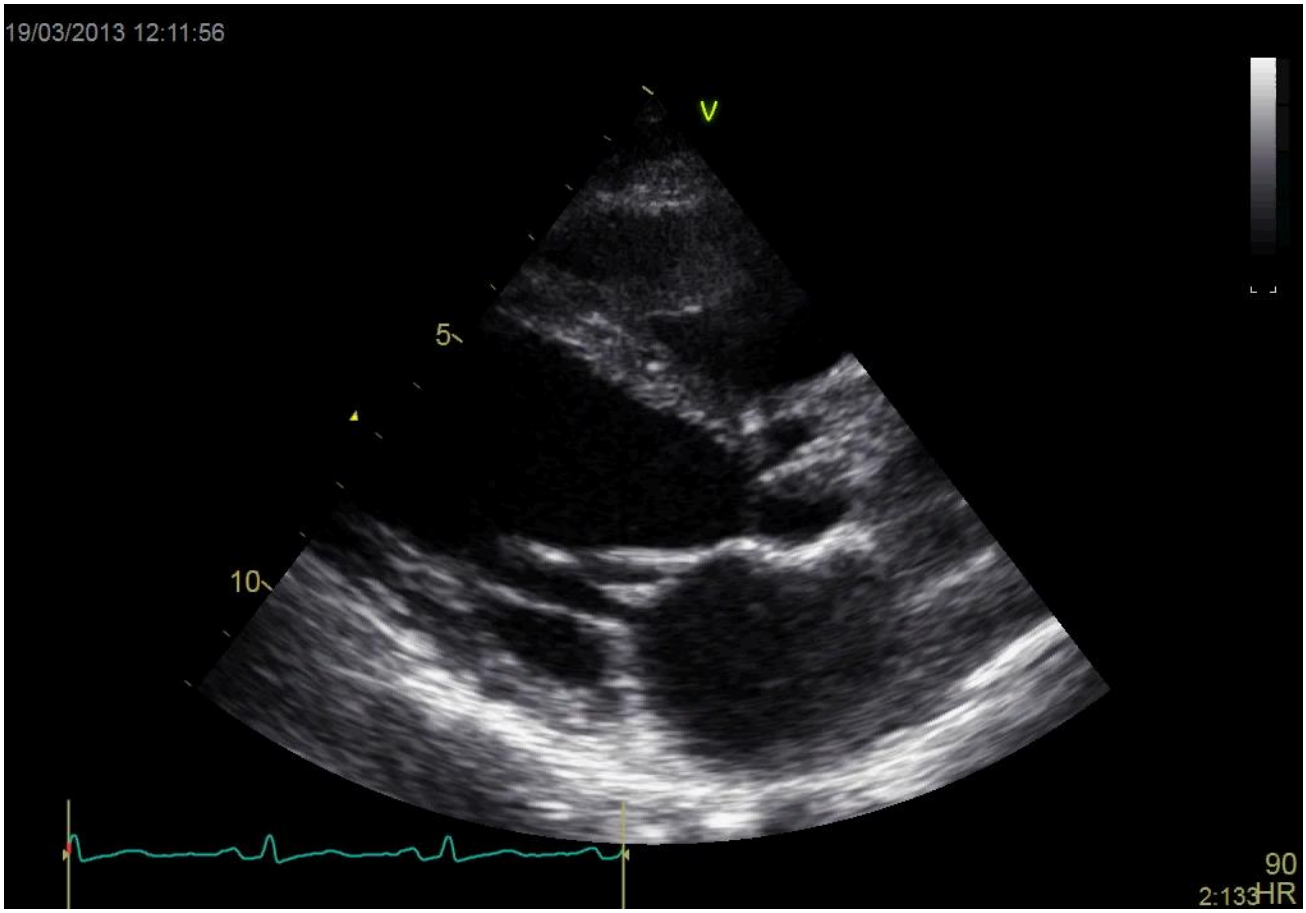
1.8. Tables

Table 1. 1. The abridged World Heart Federation diagnostic screening criteria for rheumatic heart disease

For definite RHD (either A,B,C or D)
A: Pathological MR and ≥ 2 morphological features of RHD of the MV
B: MS (mean gradient ≥ 4mmHg)
C: Pathological AR and ≥2 morphological features of RHD of the AV
D: Borderline disease of both the MV and AV
For borderline RHD (either A, B or C)
A: ≥2 morphological features of RHD of the MV without pathological MR or MS
B: Pathological MR
C: Pathological AR
Echocardiographic criteria for pathological regurgitation
Doppler echocardiographic criteria for MR (all four must be met)
1. Seen in two views
2. In at least one view, jet length ≥ 2cm
3. Velocity ≥3m/s for one complete envelope
4. Pan-systolic jet in at least one envelope
Doppler echocardiographic criteria for AR (all four must be met)
1. Seen in two views
2. In at least one view, jet length ≥ 1cm
3. Velocity ≥3m/s for one complete envelope
4. Pan-diastolic jet in at least one envelope
Echocardiographic criteria for morphological features of RHD
Features in the MV
• AMVL thickening ≥ 3mm
• Chordal thickening
• Restricted leaflet motion
• Excessive leaflet tip motion during systole
Features in the AV
• Irregular or focal thickening
• Coaptation defect
• Restricted leaflet motion
• Prolapse
RHD: Rheumatic heart disease MR: Mitral regurgitation AR: Aortic regurgitation
MV: Mitral valve AV: Aortic valve AMVL: Anterior mitral valve leaflet MS: Mitral stenosis

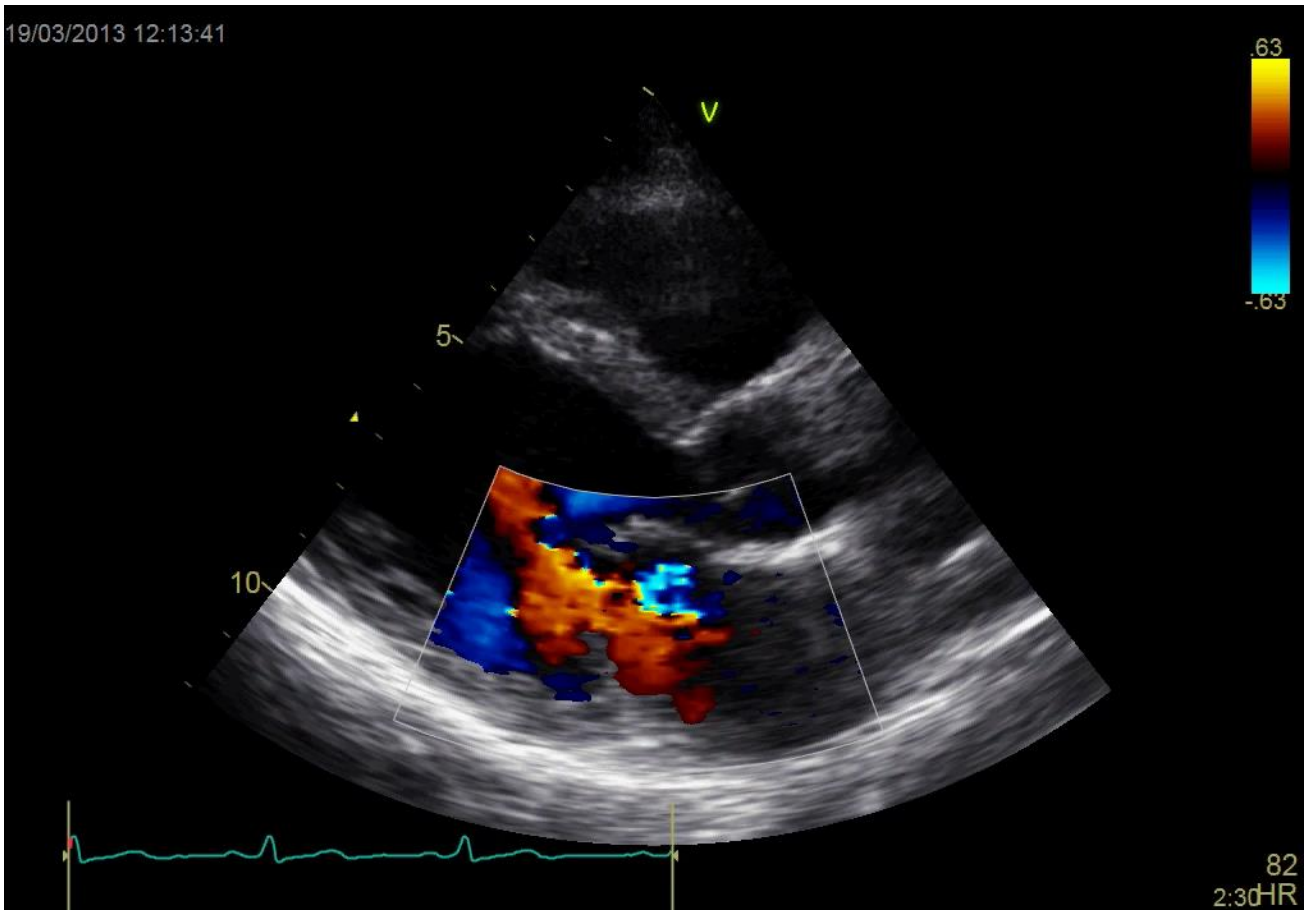
1.9. Media clips

Media clip 1. 1. Parasternal long-axis view of a mitral valve with typical RHD morphological features



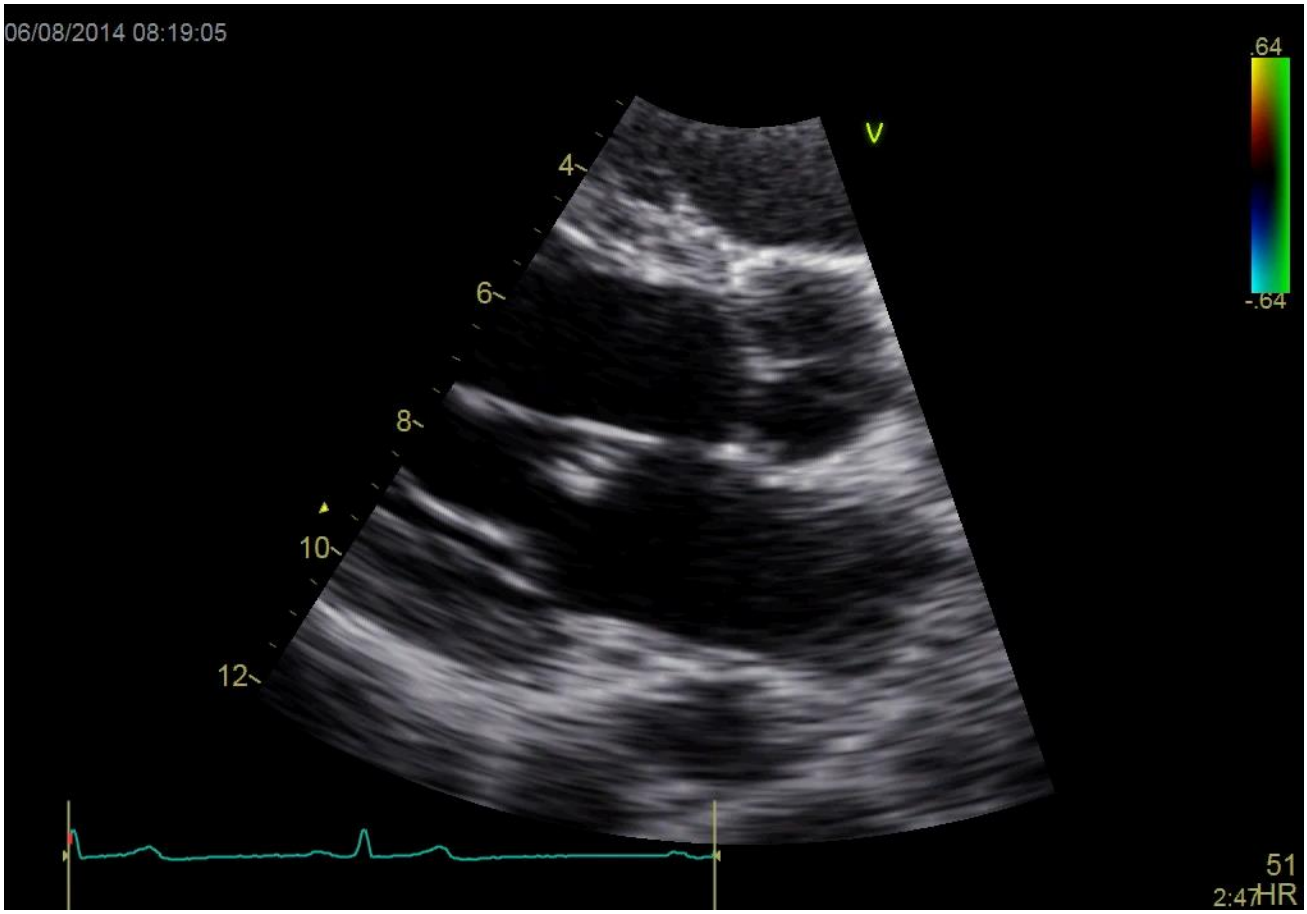
Cine-loop taken from a screening 2D echocardiogram in a parasternal long-axis view (PSLAX). There are typical morphological features of RHD of the mitral valve (diastolic restriction of both leaflets with thickening of the leaflet tips).

Media clip 1. 2. Parasternal long-axis view of the case in Media clip 1.1. with focused colour Doppler over the mitral valve.



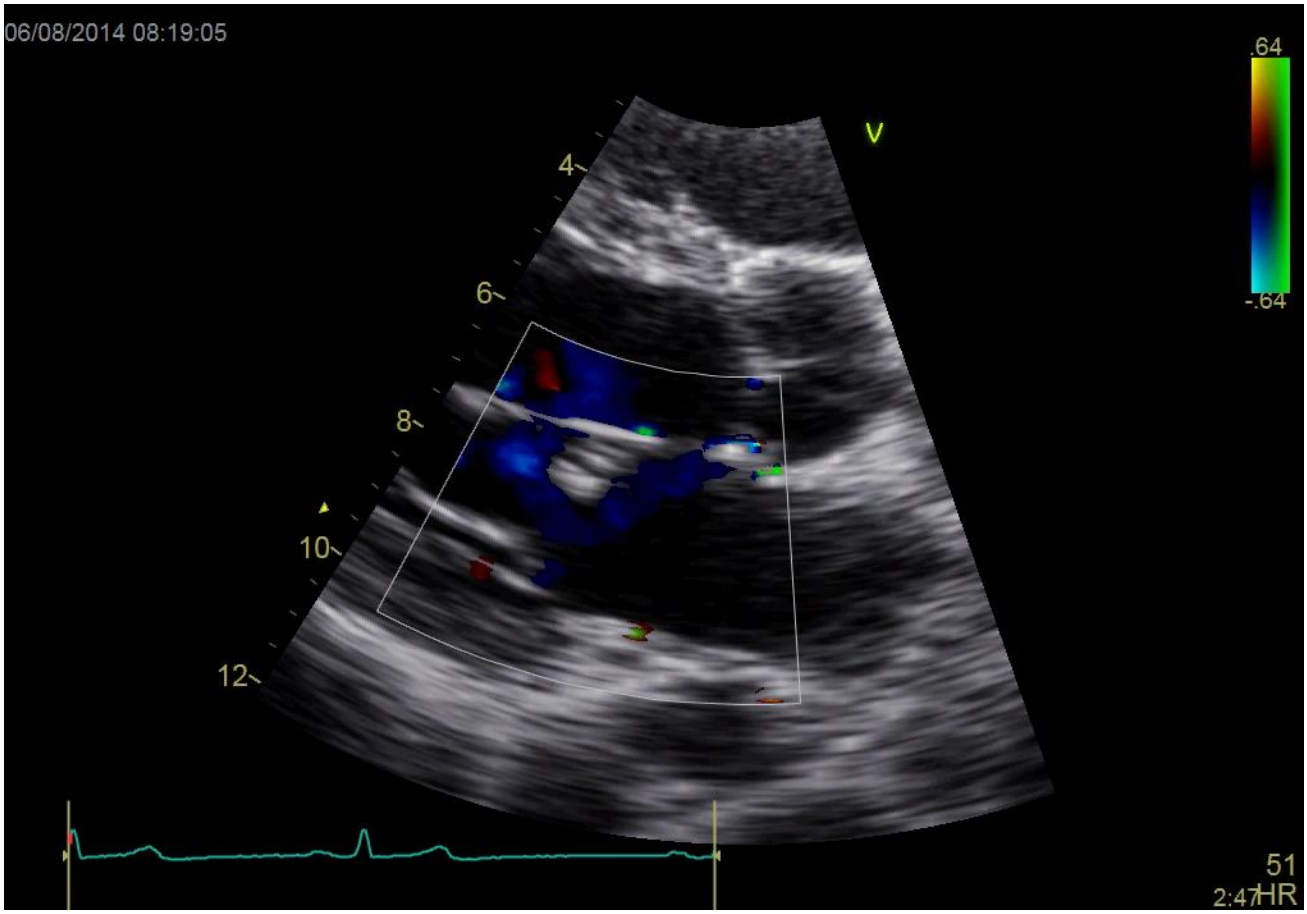
Cine-loop of corresponding case in Media clip 1.1. with focused colour Doppler over the mitral valve. There is a pixel of pixel mitral regurgitation during ventricular systole. The regurgitant jet measured <2cm and therefore the case was designated as WHF 'borderline RHD'.

Media clip 1. 3. Parasternal long-axis view of a normal mitral valve



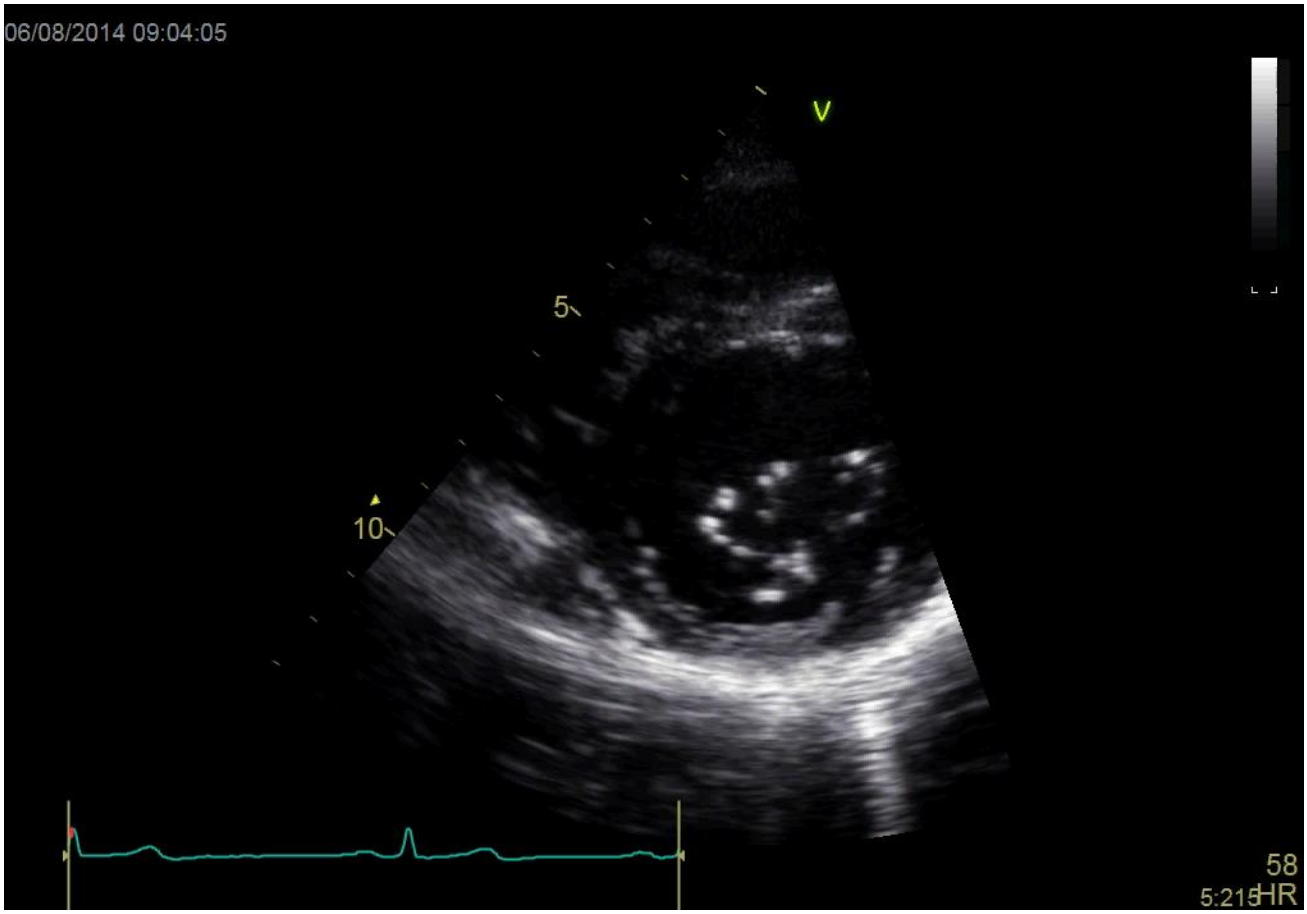
There are no morphological features of RHD of the mitral valve(both leaflets are thin and demonstrate no diastolic restriction).

Media clip 1. 4. Parasternal long-axis view of the case presented in Media clip 1.3. with focused colour Doppler over the mitral valve.

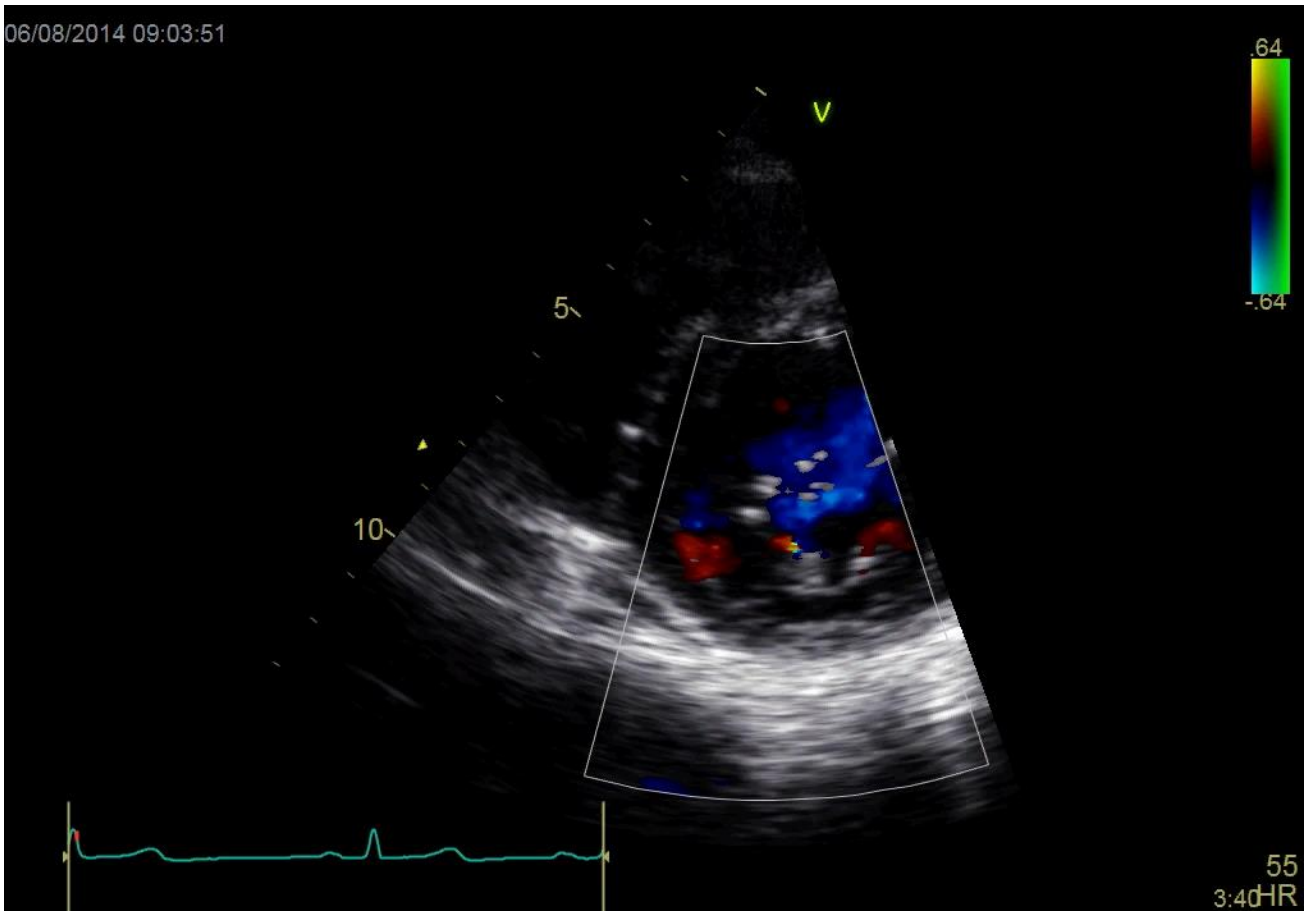


There is WHF ‘pathological’ mitral regurgitation. The regurgitant jet measured >2 cm and met all additional Doppler criteria. The screened case is therefore designated as ‘borderline RHD’.

Media clip 1. 5. Parasternal-short axis view of the case presented in Media clip 1.3 demonstrating an inter-scallop separation of the posterior mitral valve leaflet.



Media clip 1. 6. Parasternal short-axis view of the case presented in Media clip 1.3 with focused colour Doppler over the mitral valve.



A typical example of inter-scallop (ISS)-related mitral regurgitation is presented in this case. Here, the regurgitant jet is appreciated at the ISS and is seen to be moving in a vertical up-down fashion through the posterior mitral valve leaflet (PMVL).

Chapter 2: Inter-scallop separations of the posterior leaflet of the mitral valve- an important cause of 'pathological' mitral regurgitation in rheumatic heart disease screening

Chapter two consists of a publication featuring the role of inter-scallop separations (a feature of a normal mitral valve) in inflating the WHF 'borderline RHD' category in a population screened for subclinical RHD. I am the principal author of the article. MJ Monaghan, GW Lloyd and AJK Pecoraro reviewed the final draft of the manuscript. AF Doubell and PG Herbst were the co-supervisor and supervisor respectively. Both reviewed the final draft of the manuscript.

Published manuscript

Inter-scallop separations of the posterior leaflet of the mitral valve: an important cause of 'pathological' mitral regurgitation in rheumatic heart disease screening
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Echo Res Pract. 2018;5(2):29-34. doi:10.1530/ERP-18-0010

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2.1. Summary

The 2012 World Heart Federation (WHF) criteria for echocardiographic diagnosis of rheumatic heart disease (RHD) identify that the finding of 'pathological' mitral regurgitation (MR) in a screened individual increases the likelihood of detecting underlying RHD. Cases of isolated 'pathological' MR are thus identified as 'borderline RHD'. A large-scale echocardiographic screening program (Echo in Africa) in South Africa has identified that

inter-scallop separations of the posterior mitral valve leaflet (PMVL) can give rise to 'pathological' MR. The authors propose that this entity in isolation should be identified and excluded from the WHF 'borderline RHD' category. In this case report, we present two examples of 'pathological' MR related to inter-scallop separation from the Echo in Africa image database. We further provide screening tips for the accurate identification of this entity.

2.2. Learning points

- Posterior mitral valve leaflet inter-scallop separations are an important entity to identify as a potential cause of haemodynamically insignificant yet WHF 'pathological' MR.
- Cases of inter-scallop separations with 'pathological' MR remain an important finding in RHD screening and those without any other morphological features of RHD should be excluded from the WHF 'borderline RHD' group.
- Careful interrogation of the mitral valve in both PSLAX and PSSAX views is required to identify the underlying mechanism of MR
- Further study is needed in our study population to describe the prevalence and clinical relevance of inter-scallop separations

2.3. Background

Rheumatic heart disease (RHD) is responsible for significant morbidity and mortality in developing nations and underserved communities in the developed world.[1] Sub-Saharan Africa has been identified as an area with endemic RHD with an estimated 10 per 1000 population living with RHD.[2]

The consensus-derived diagnostic criteria established in 2012- the 'World Heart Federation criteria for echocardiographic diagnosis of rheumatic heart disease'[3] represent an important milestone in the standardization of diagnostic parameters and the reporting of RHD. However, the criteria are potentially weakened by the incorporation of a non-specific Doppler-based evaluation of regurgitant valvular lesions that could erroneously include cases with "congenital mitral regurgitation" into the 'borderline RHD' group.[4-7] A large-scale RHD screening program in both high- and low-risk RHD communities in the Western Cape, South

Africa (unpublished data from the Echo in Africa (EIA) program) has identified that a normal variant of the posterior mitral valve leaflet(PMVL) - so called 'prominent posterior leaflet inter-scallop separation'[5] may be responsible for a proportion of screened cases identified as 'borderline RHD' with isolated WHF 'pathological' MR. These separations, "*indentations*" or "*slits*" have been described in anatomical cardiology texts dating back to the 1950's .[8-11] They are known to exclusively affect the PMVL with marked heterogeneity relating to their location, number (between 0-5 separations in a single PMVL) and depth of excursion into the PMVL.[8,11] V*ictor* and *Nayak* propose in their autopsy series that these separations are a normal finding in the PMVL and play an important role in allowing the PMVL to "*change in contour and size during atrial and ventricular systole"*[11] To the best of our knowledge, there is no echocardiographic data that highlight this entity and document its particular relevance to RHD screening.

2.4. Case presentation

We present two cases selected from the EIA database of 'borderline RHD' with isolated 'pathological' MR attributable to prominent inter-scallop separations of the PMVL. Both cases met all 4 Doppler criteria for WHF 'pathological' MR.

Investigation

Participants in the Echo in Africa program are screened by experienced RHD sonographers using the General Electric (GE) Vivid I ™ laptop unit. Screening studies are captured in accordance with a minimum standard dataset as stipulated by the British Society for Echocardiography[12].

1. **Case 1** is a screening study obtained from an individual living in a high risk RHD community (low socio-economic status (SES) and with no access to private medical care). Media clip 2. 1, Media clip 2. 2, Media clip 2. 3, Media clip 2. 4 have been selected from the initial screening study to demonstrate the relevant findings.

2. **Case 2** is from an individual living in a low risk RHD community (high SES with access to private medical care). Media clip 2. 5, Media clip 2. 6, Media clip 2. 7, Media clip 2. 8 have been selected from the initial screening study to demonstrate the relevant findings.

2.5. Tips for the echocardiographic diagnosis of inter-scallop separations in screening

Parasternal long axis

- Dynamic scanning with colour Doppler is used to identify the segment of the PMVL where the regurgitant jet is seen to be maximal and thus coinciding with the inter-scallop separation. The so called 'parasternal sweep' is performed by tilting the probe up (more lateral portion – P2/P1) and then tilting downwards (more medial portion [P2/P3]; Figure 2. 1. The parasternal sweep)

- Suspect an inter-scallop separation as a cause of MR particularly when the MR jet is centrally directed and shown to emanate from below the coaptation point of the PMVL with the anterior mitral valve leaflet (AMVL)- see Media clip 2. 9. This feature however cannot be solely relied upon as regurgitant jets can be posteriorly directed (see Media clip 2. 2)and can therefore mimic the classic 'pseudoprolapse' mechanism of chronic rheumatic MR in terms of jet direction (see Media clip 2. 10, Media clip 2. 11).

Parasternal short axis

- Tilt to view leaflet tips of PMVL without colour – try to identify the separate scallops and the separations that demarcate them (see Media clip 2. 12). Inter-scallop separations are identified as visible linear defects extending a variable depth into the posterior leaflet from the coaptation line. The inter-scallop area is often seen to 'open up' during diastole when the scallop edges part in this region.

- Colour Doppler is used to confirm site of regurgitation (often below coaptation line of the two mitral leaflets)- see Media clip 2. 13. The colour jet origin is typically confined to the inter-scallop region and the jet origin spreads predominantly vertically down the height of the PMVL rather than across the coaptation line between the AMVL and PMVL.

2.6. Discussion

In this case report we describe a known anatomical feature of the PMVL, so-called inter-scallop separation, that can be attributed to cause WHF 'pathological' MR in screened cases from both a high- and low risk RHD community.

Identifying alternative 'congenital' causes of mitral regurgitation is an important step in the screening process and specifically stipulated by the WHF in an attempt to curb misidentification of non-rheumatic cases. Included amongst these are congenital anomalies related to leaflet clefts or apparent clefts. Amongst the mimics of mitral leaflet clefts are entities that can be readily differentiated from true clefts such as the trileaftet mitral valve. In this entity the identification of an additional papillary muscle aids in diagnosis.[13] True, isolated AMVL clefts (not related to atrio-ventricular septal defects) are a rare finding,[14,15] but due to an absence of anatomical scallops of the AMVL, these entities are not confused with inter-scallop separations and readily identified as congenital abnormalities

The same does not hold true for cleft-like defects identified in the PMVL. As early as the 1950's there was contention as to what constitutes the PMVL and importantly, no consistent anatomical nomenclature was agreed on to define its variable divisions.[8,10,11,16] This lack of agreement persists and there is no consensus on what features differentiate the normal anatomical variant of an inter-scallop separation from a cleft or even whether a separation of the entities is warranted. Two strategies of identifying true clefts from amongst inter-scallop separations have been to look as either the size of the defect or functional consequence thereof. A recent study describing the prevalence and impact of clefts in mitral valve prolapse (MVP) identified clefts as defects that extend >50% of the height of the posterior leaflet and are visible during systole and diastole.[17] The authors conclude that these clefts may play an important role in the development and mechanism of prolapse but acknowledge that they likely reflect one end of the spectrum of normality, having also been identified in their control population.[17]
In contrast to this, *Wyss et al*. propose that a cleft is defined as a *"complete split up to the annulus and has some degree of regurgitation"*[18], choosing instead to incorporate a functional deficit into the definition.

The addition of this functional aspect does not appear to be strongly rooted in a fundamental difference in pathology identified and it is our contention PMVL clefts, something previously considered rare,[19-21]is perhaps only one end of the spectrum of normal inter-scallop separation. [17]

This raises important challenges in the RHD screening environment. Whereas the impact of PMVL 'clefts' have been explored in cohorts with overt valve pathology their role remains undefined in healthy asymptomatic children undergoing RHD screening. Inter-scallop separation, a normal anatomical entity, has not been identified as a potential confounder in the current WHF diagnostic criteria. Consequently, if not recognized and excluded from analysis, could falsely increase the prevalence of 'borderline RHD' detected in any RHD screening program. In addition, failure to exclude these cases could further distort the findings of long term studies documenting the outcome of 'borderline RHD' as progression of MR from this entity remains unexplored.[22,23,24]

Therefore, in the absence of compelling RHD-related morphological changes of the AMVL or the aortic valve (AV), we do not advocate diagnosing cases with RHD where the mechanism of WHF 'pathological' MR is clearly related to an inter-scallop separation. To what extent these cases are currently inflating the size of the borderline group is an important question that needs to be further studied.

Given the ubiquitous nature of PMVL inter-scallop separations, it is inevitable that screening programs will encounter individuals with true RHD features of their mitral valve in addition to their normal variation of the PMVL (inter-scallop separation with mitral regurgitation). What remains unclear is what specific features of the mitral valve in cases of 'presumed normal PMVL variants' will alert the screener to consider a diagnosis of concurrent RHD.

Further research is required to describe the prevalence and natural history of inter-scallop separations in both high- and low risk RHD populations and the effect that the RHD process has on this underlying normal PMVL variant.

Funding statement

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Declaration of interest

The authors declare no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

Patient consent

The Echo in Africa program has received ethical approval from the Health Research Ethics Committee of Stellenbosch University (N14/04/038).

Participants in this study are required to provide adequate assent/consent prior to study enrollment. This includes the use of relevant images/data for publication. Studies are de-identified prior to data analysis to ensure participant anonymity.

Author contributions and acknowledgements

LH screened the participants and wrote the manuscript with input from all the authors. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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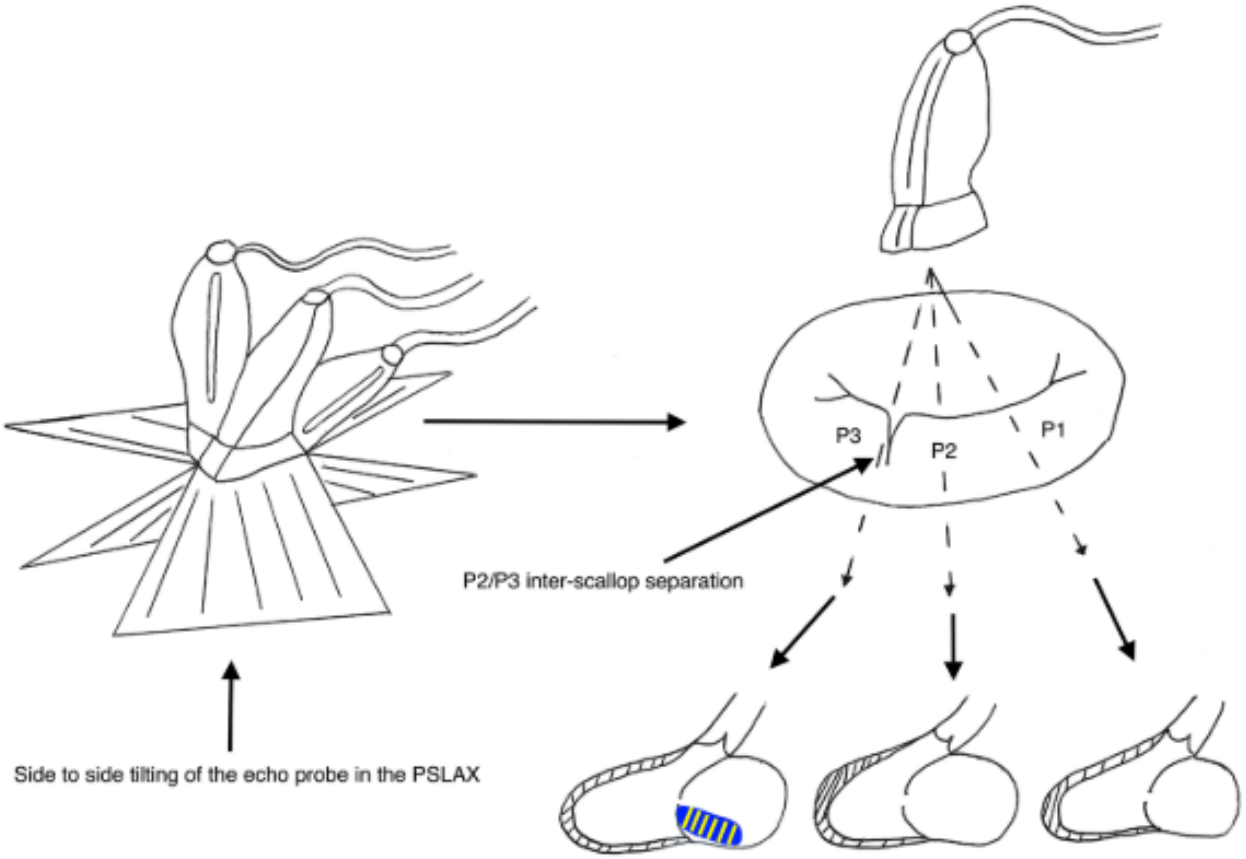
2.8. Figures

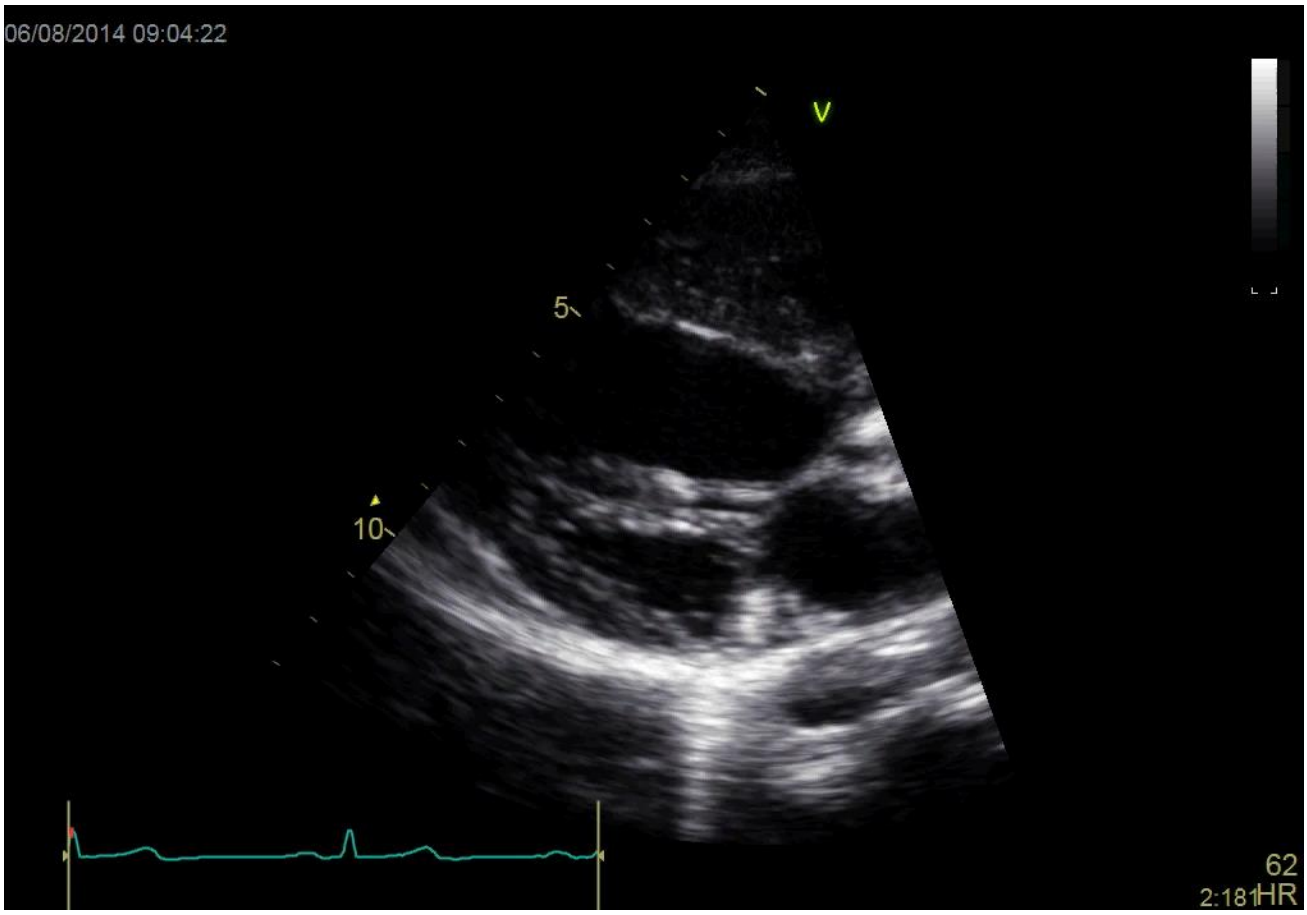
Figure 2. 1. The parasternal sweep



The parasternal sweep is performed by sweeping from commissure to commissure (tilting the echo probe from side to side in the PSLAX) with focused colour Doppler over the MV will identify, which segment is likely to have an inter-scallop separation.

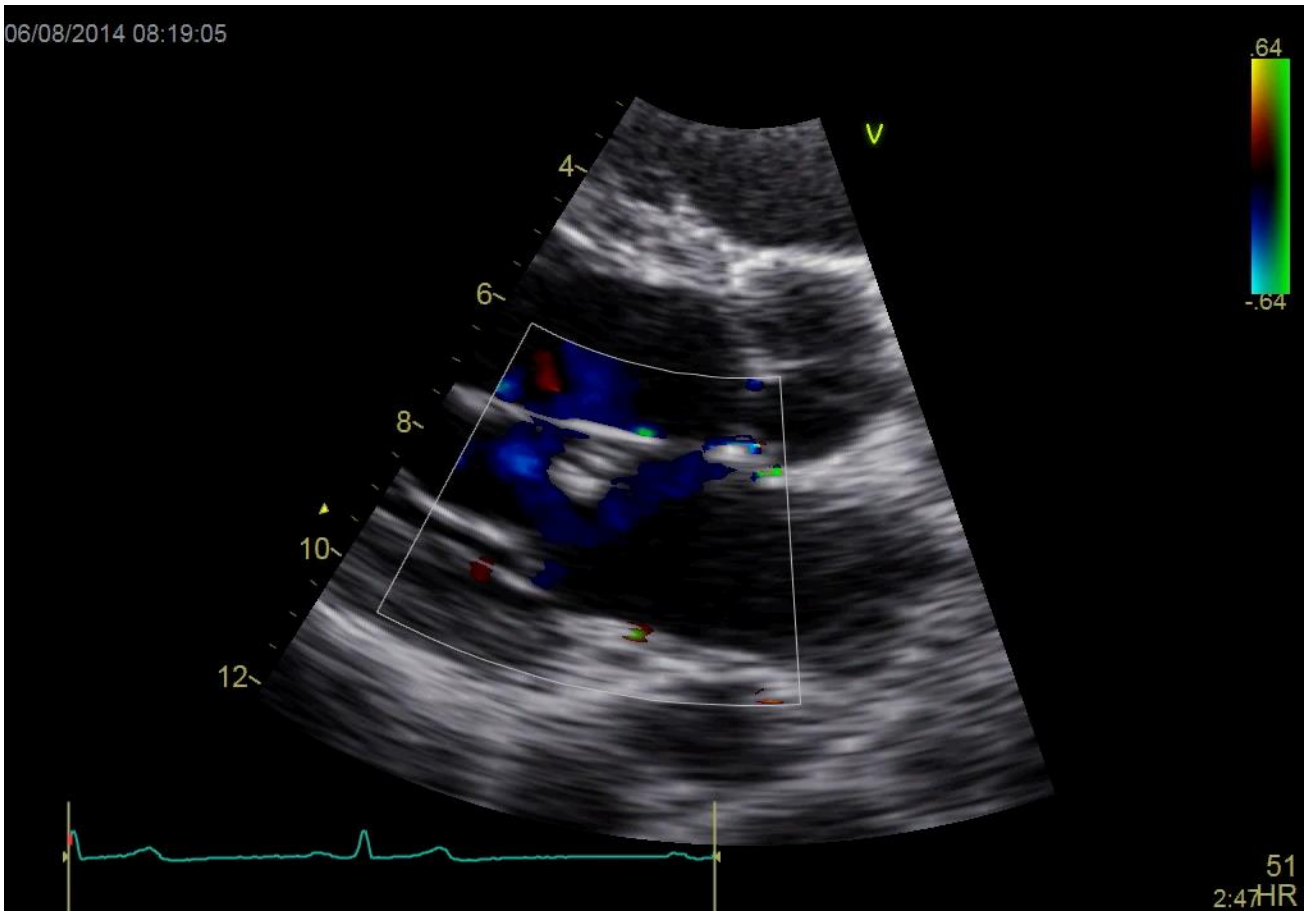
2.9. Media clips

Media clip 2. 1. Parasternal long-axis view a normal mitral valve.



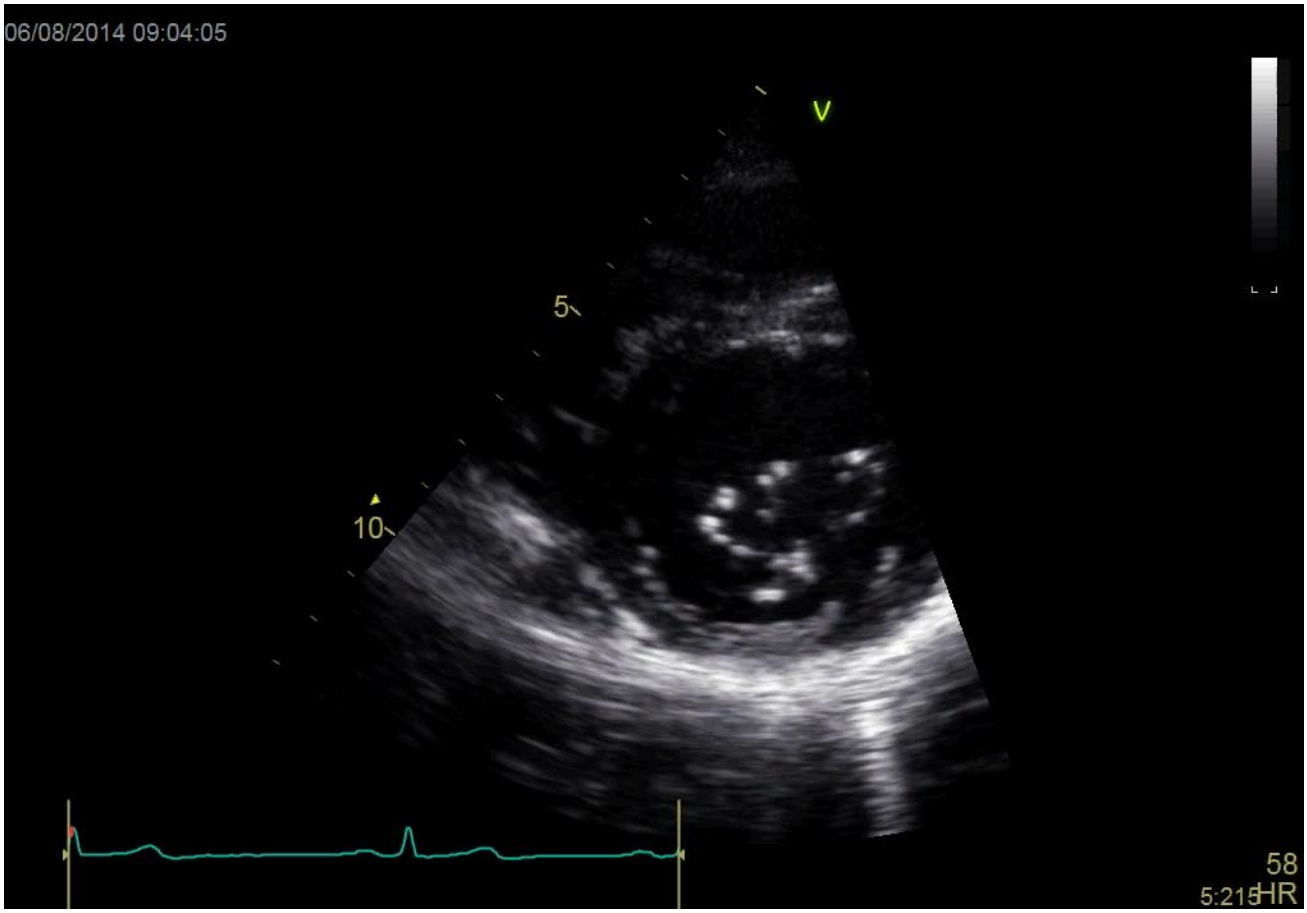
A normal mitral valve (normal anterior mitral valve leaflet thickness with no rheumatic-related restriction of either mitral valve leaflets.

Media clip 2. 2. Parasternal long-axis view of the case presented in Media clip 2.1. with focused colour Doppler over the mitral valve.



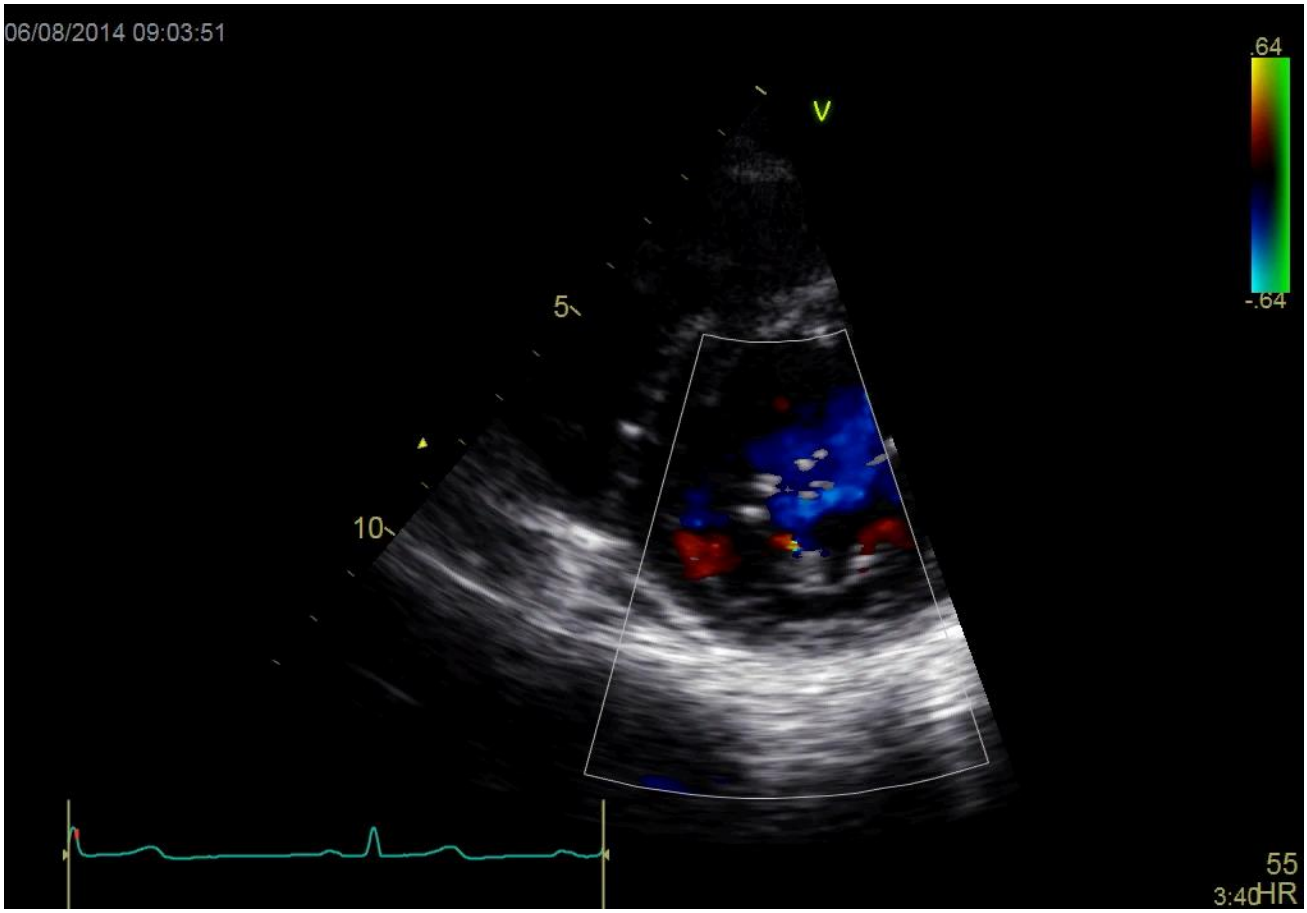
There is World Heart Federation (WHF) ‘pathological’ mitral regurgitation (MR). The regurgitant jet measured >2 cm and met all additional Doppler criteria. The screened case is therefore designated as WHF ‘borderline RHD’.

Media clip 2. 3. Parasternal short-axis view of the case presented in Media clip 2.1.



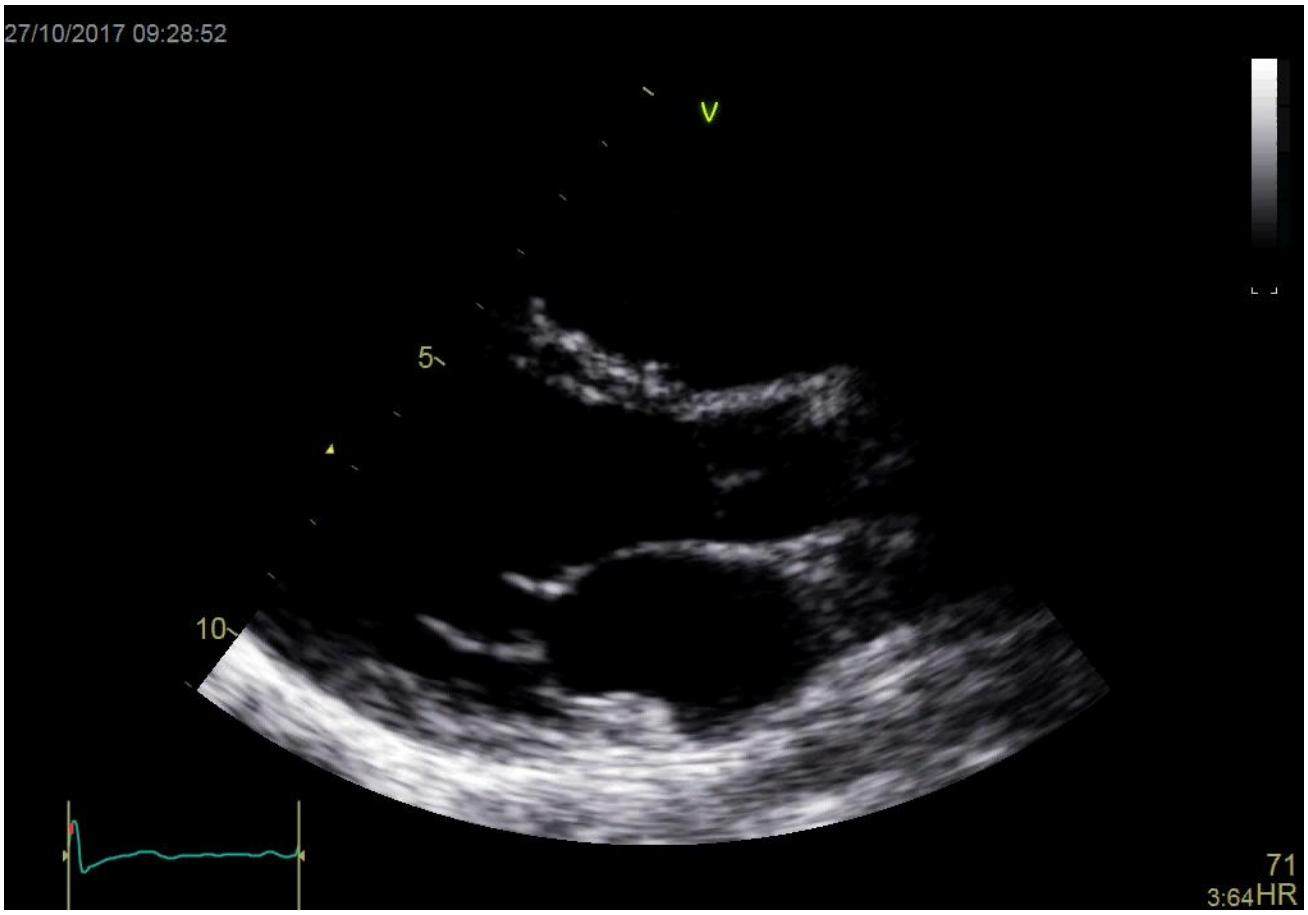
An inter-scallop separation (ISS) of the posterior mitral valve leaflet (PMVL) in the P2 position.

Media clip 2. 4. Parasternal short-axis view of the case presented in Media clip 2.1. with focused colour Doppler over the mitral valve



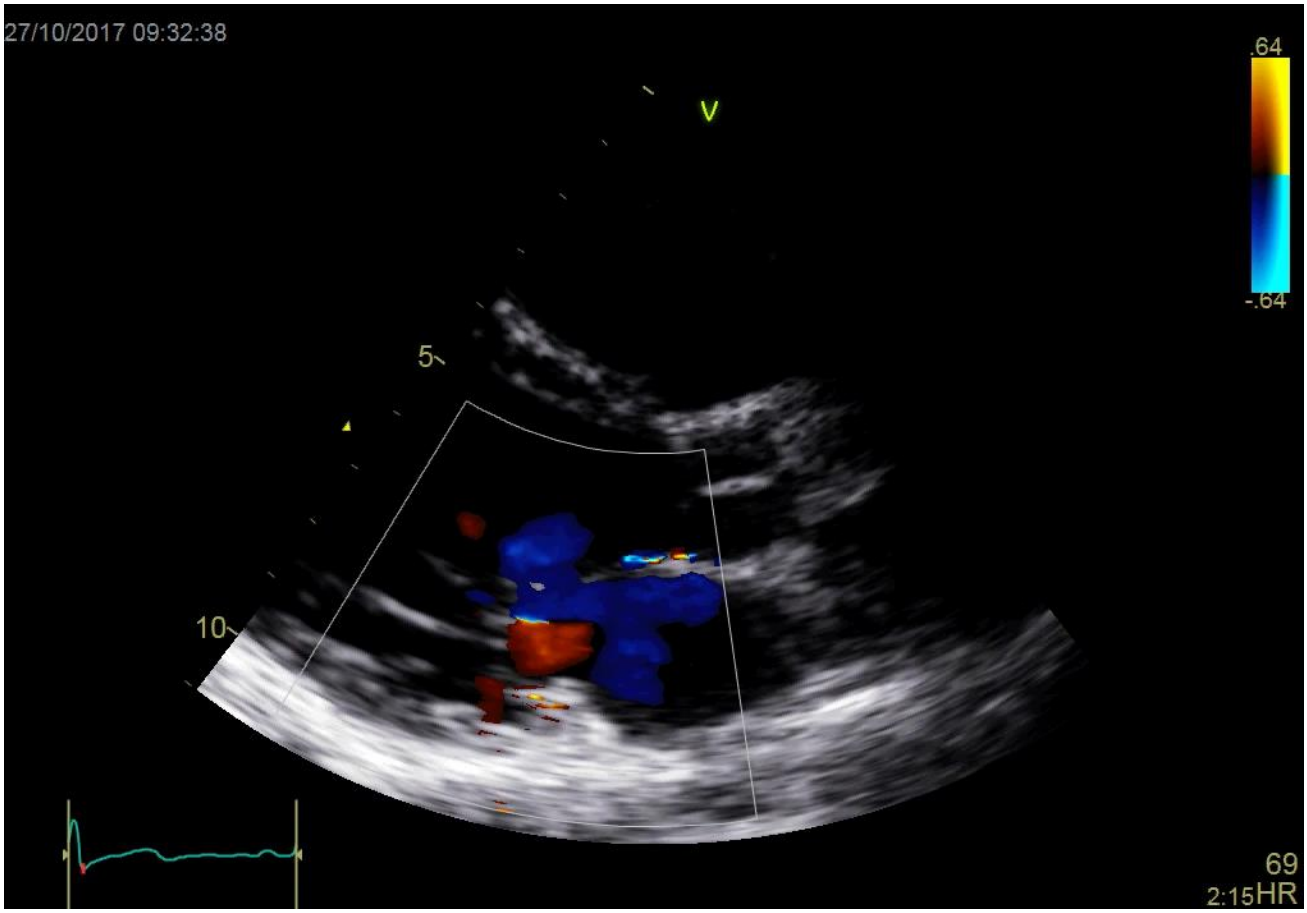
Colour Doppler over the MV confirms a P2 ISS as the underlying cause of the MR.

Media clip 2. 5. Parasternal long-axis view of a normal mitral valve



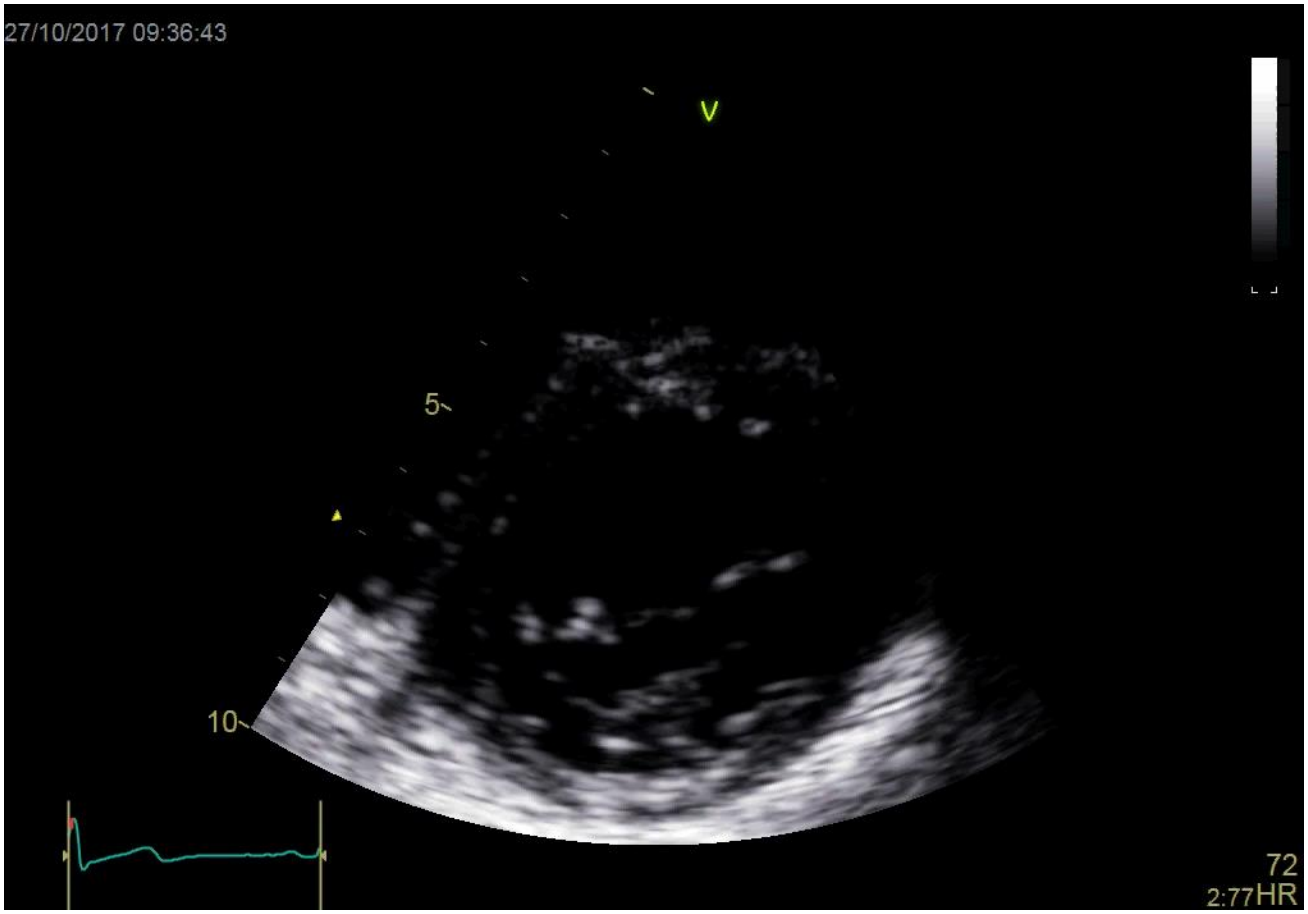
A normal MV (normal anterior mitral valve leaflet thickness with no rheumatic-related restriction of either MV leaflets.

Media clip 2. 6. Parasternal long-axis view of the case presented in Media clip 2.5. with focused colour Doppler over the mitral valve.



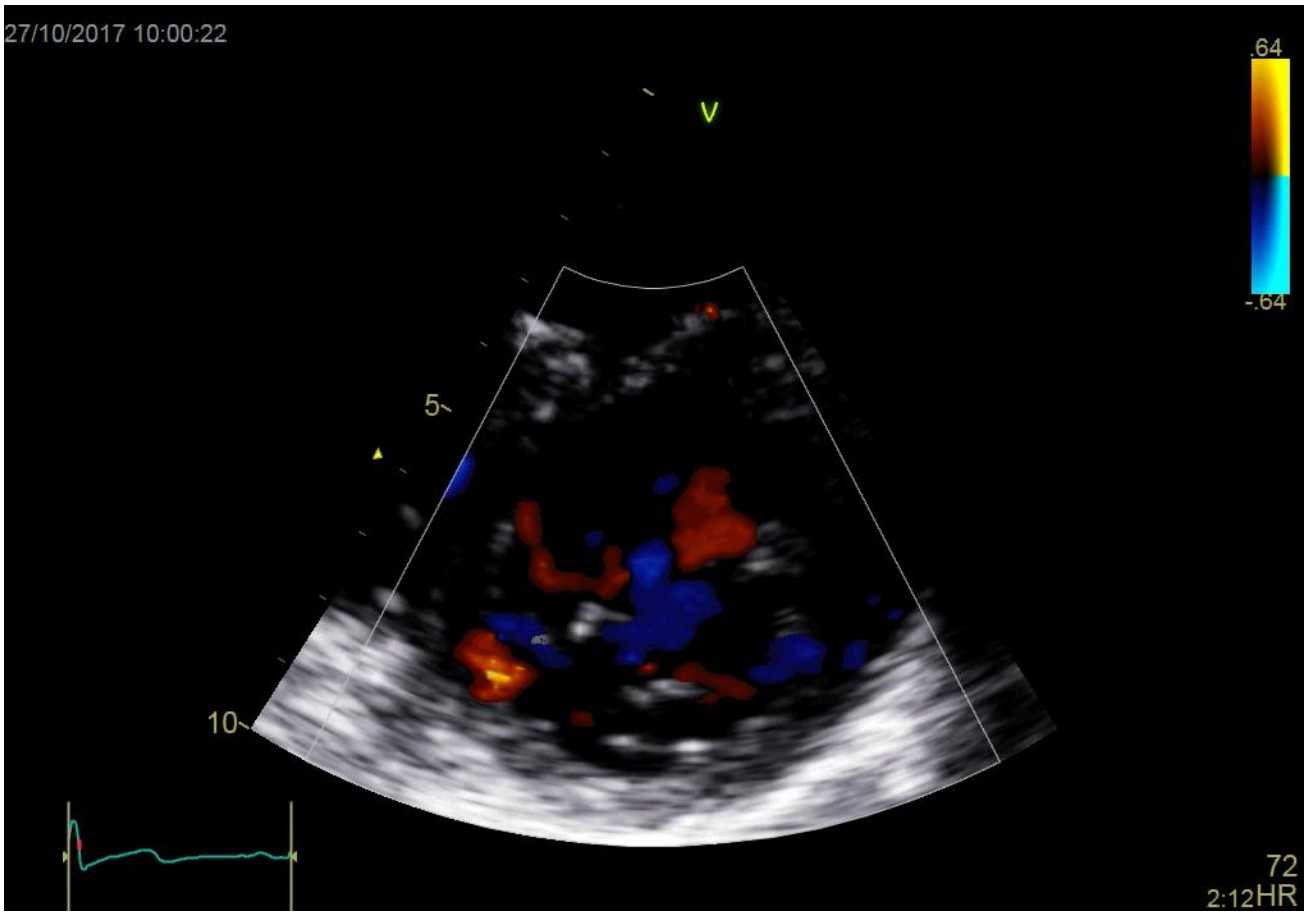
There is WHF ‘pathological’ MR. The regurgitant jet measured >2 cm and met all additional Doppler criteria. The screened case is therefore designated as ‘borderline RHD’.

Media clip 2. 7. Parasternal short-axis view of the case presented in Media clip 2.5.



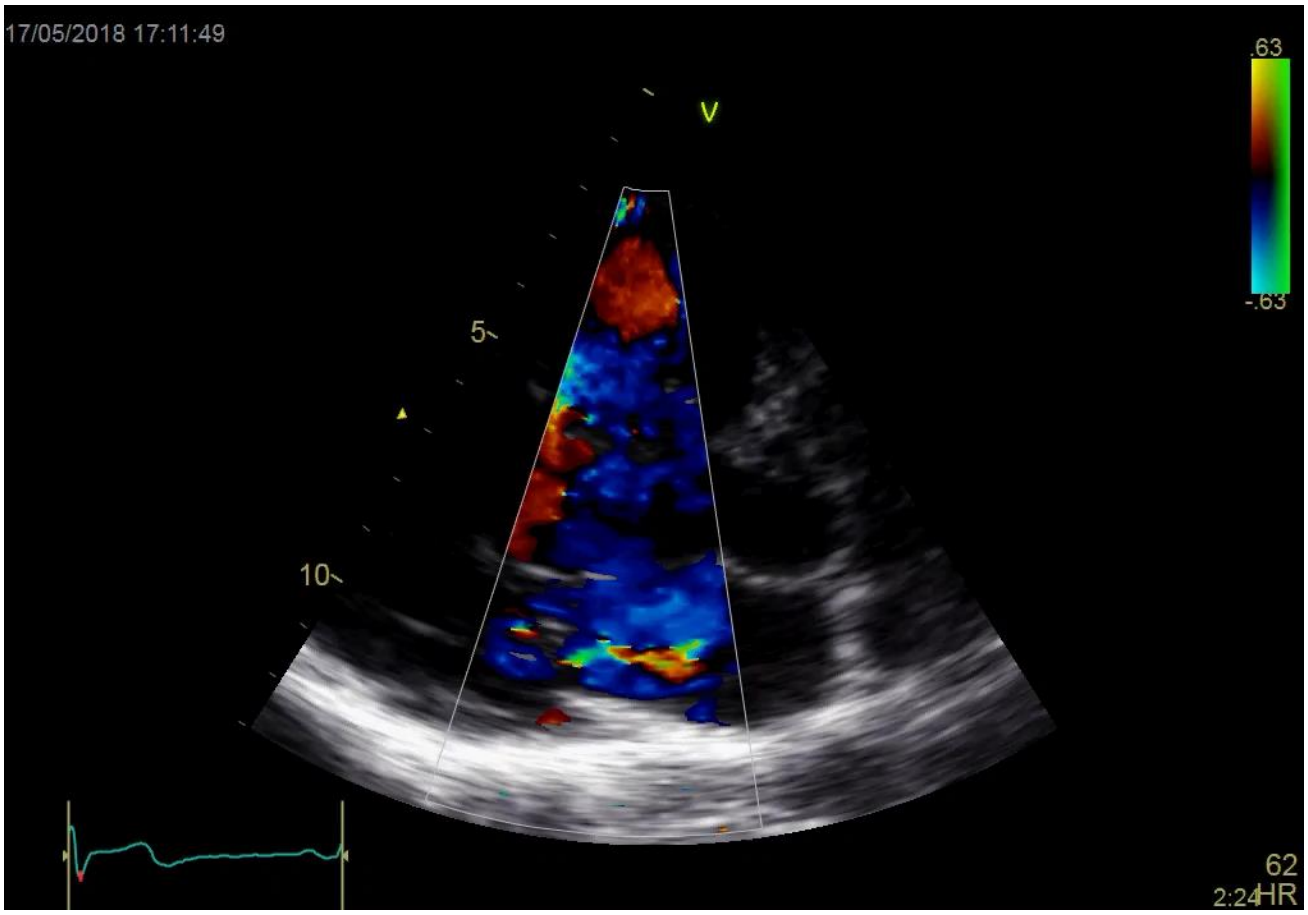
A subtle case of an ISS in the P2/P3 position is appreciated

Media clip 2. 8. Parasternal short-axis view of the case presented in Media clip 2.5. with focused colour Doppler over the mitral valve.



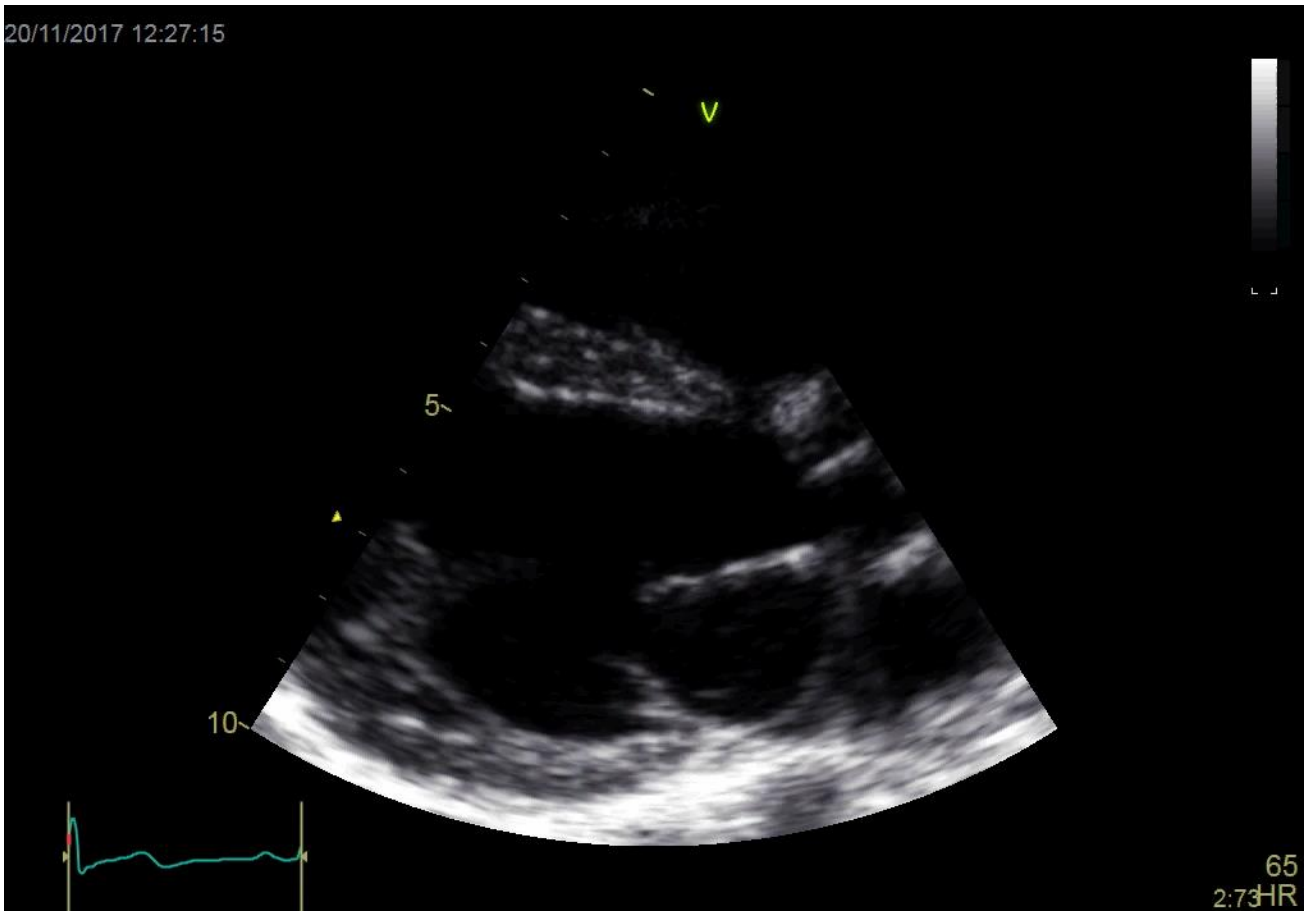
An example of ISS-related MR is presented in this case. Here, the regurgitant jet is appreciated as a ‘spot’ of colour over the P2/P3 ISS.

Media clip 2. 9. Parasternal long-axis view of a mitral valve with ‘pathological’ MR



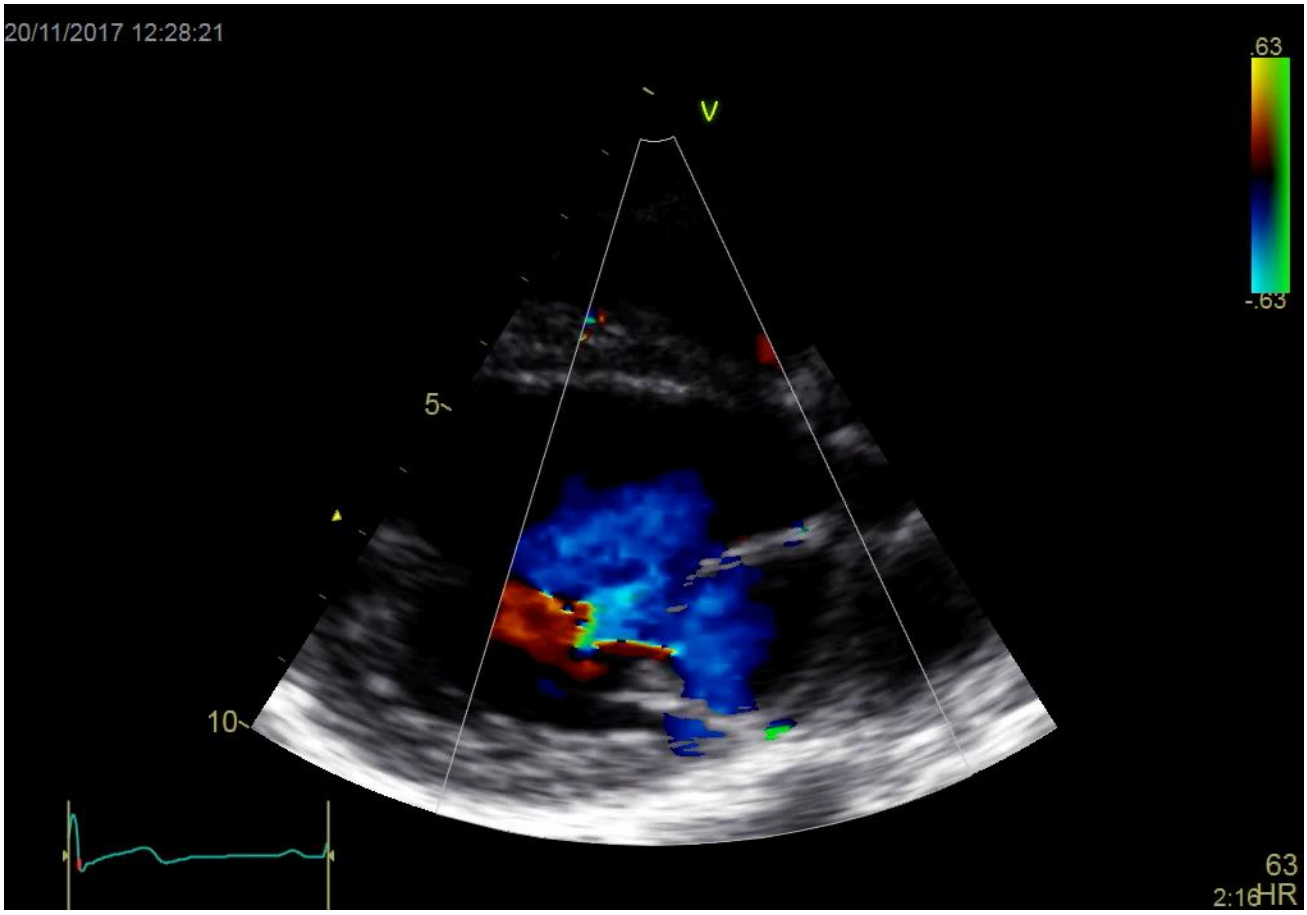
A screened case from the EIA database with ‘pathological’ MR from an ISS of the PMVL. Colour Doppler over the MV demonstrates central MR with its origin below the coaptation point of the MV leaflets (i.e. body of the PMVL).

Media clip 2. 10. Parasternal long-axis view of a rheumatic mitral valve



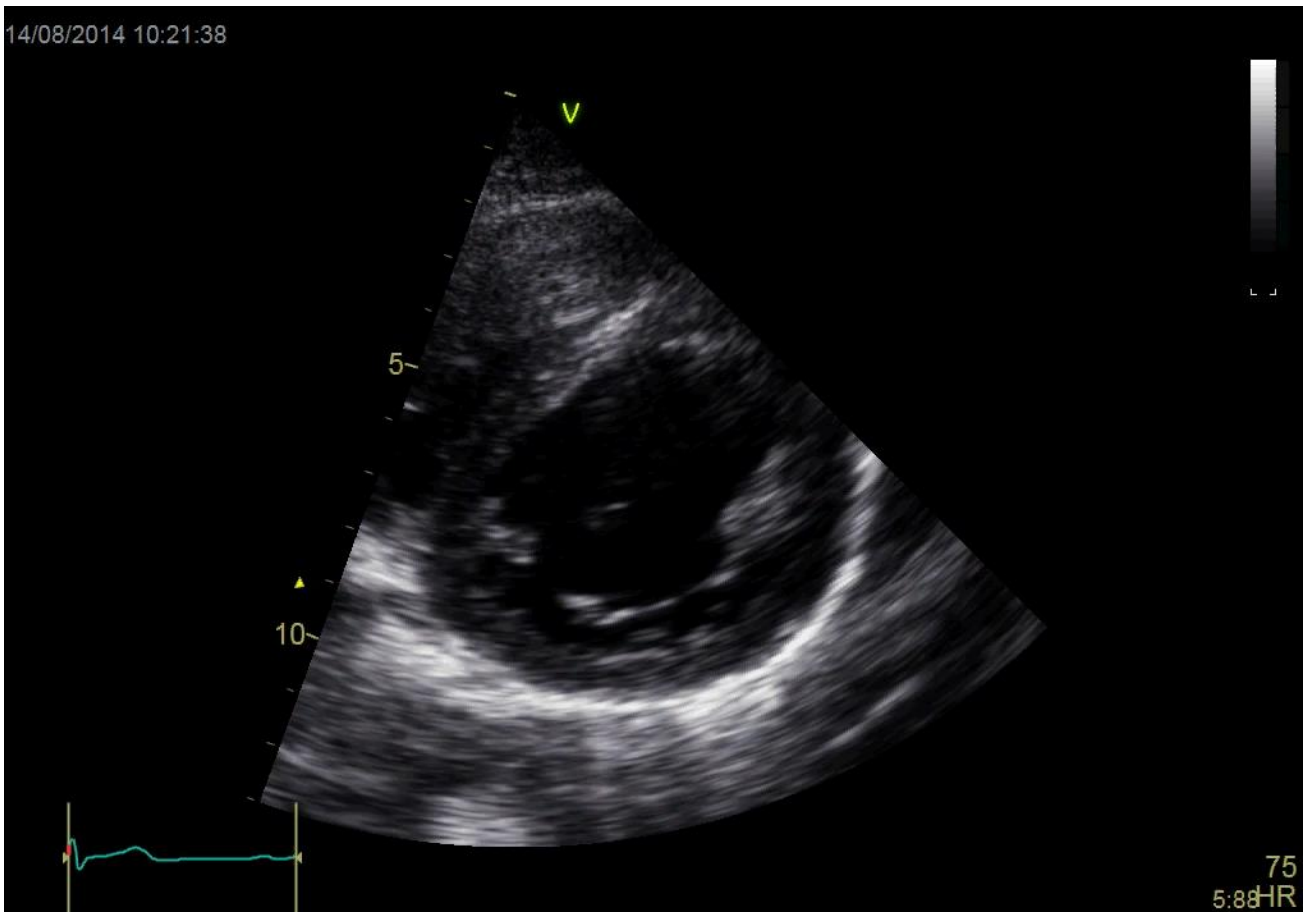
There is suggestive rheumatic-related restriction of the AMVL and PMVL. The A2 segment of the AMVL is seen to 'prolapse' past the P2 segment of the PMVL. This mechanism is more correctly termed 'pseudoprolapse' as the AMVL is in its normal position at end systole. The impression of A2 prolapse is rather thought to be related to PMVL systolic restriction with resultant malcoaptation of the PMVL and AMVL during systole. This generates the characteristic posteriorly directed jet of rheumatic mitral regurgitation.

Media clip 2. 11. Parasternal long-axis view of the case presented in Media clip 2.10.



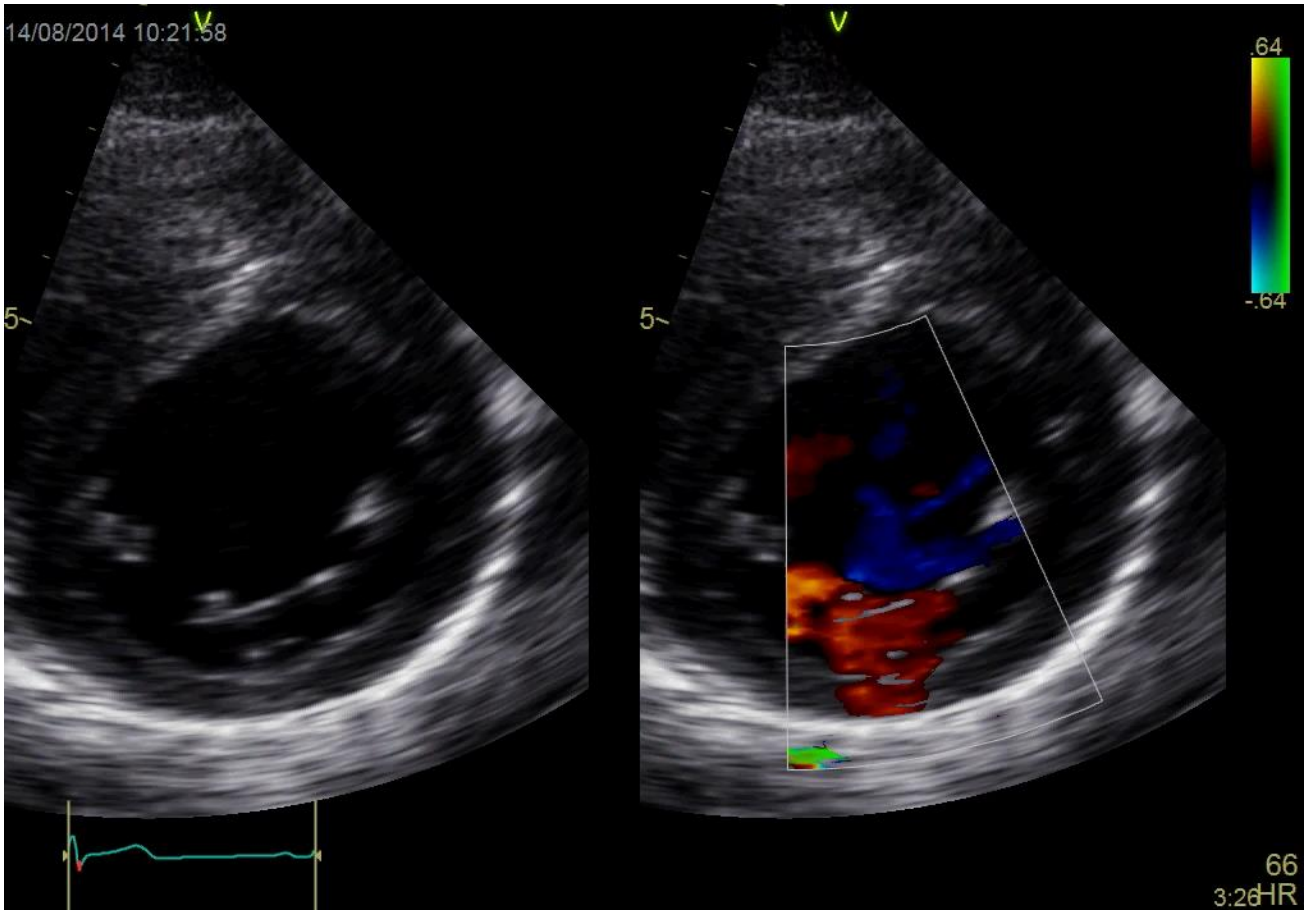
Colour Doppler over the MV demonstrates the characteristic posteriorly directed MR jet encountered in chronic rheumatic MR

Media clip 2. 12. Parasternal short axis view of the mitral valve in a screened Echo in Africa participant with 'pathological' MR



The screener should ensure that the leaflet tips are adequately sectioned to identify potential ISS. This clip demonstrates an ISS in the P2/P3 position.

Media clip 2. 13. Parasternal -short axis view with and without focused colour Doppler of the mitral valve of the case presented in Media clip 2.12.



Colour Doppler over the MV confirms a P2 ISS as the underlying cause of the MR.

Chapter 3: Inter-scallop separations of the posterior mitral valve leaflet: a solution to the 'borderline RHD' conundrum?

Chapter three consists of a manuscript reporting on the results of a prospective cross-sectional echocardiographic study. The manuscript has been submitted to the International Journal of Cardiology. The reviewers comments have been addressed in a revised manuscript (shown here) and we await formal feedback from the Editor. My role in the study included developing the study protocol and performing and capturing all echocardiographic assessments of all enrolled study participants. I am the primary author of the manuscript included in this chapter. CJ Lombard assisted with the statistical analysis of the data. He reviewed the final draft of the manuscript. MJ Monaghan, GW Lloyd, AJK Pecoraro reviewed the final draft of the manuscript. AF Doubell and PG Herbst were the co-supervisor and supervisor respectively. They supervised the study design and execution. Both reviewed the final draft of the manuscript.

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3.1. Abstract

Introduction

The World Heart Federation (WHF) criteria incorporate a Doppler-based system to differentiate between 'physiological' and 'pathological' mitral regurgitation (MR) -a sole criterion sufficient for the diagnosis of WHF 'borderline rheumatic heart disease' (RHD). We have previously identified inter-scallop separations(ISS) of the posterior mitral valve leaflet (PMVL) as a non-rheumatic mechanism of 'pathological' MR in high-risk children. This study aimed to establish the prevalence of ISS and ISS-related MR amongst a cohort of South African children with high-and very low-RHD risk. Furthermore, we define a mechanistic approach for MR evaluation in RHD screening that may assist in the differentiation between true RHD and other non-rheumatic entities.

Methods and results

A prospective cross-sectional echocardiographic study of 759 school children (aged 13-18) was performed. ISS were identified using handheld echocardiography(HH). Cases with MR≥1.5cm on HH underwent a second comprehensive study to determine the prevalence of RHD according to the WHF guideline and establish the underlying mechanism of MR according to a predefined screening algorithm. Of 400 high-risk children, two met criteria for 'definite RHD' (5 per 1000 [95% CI, 1.4-18.0]; p=0.5) and 11 for 'borderline RHD' (27.5 per 1000 [95% CI 15.4-48.6]). There were no cases of 'definite RHD' in the very low-risk cohort (359 children). There were 14 cases of 'borderline RHD' in the very low-risk cohort (39 per 1000 [95% CI 23.4-64.4], p=0.37). ISS were identified in 278 (69.5%) children in the high-risk cohort and 269(74.9%) children in the very low-risk cohort (p=0.10) Comprehensive echocardiography identified an underlying ISS as the mechanism of isolated 'pathological' MR in 11 (2.8%) high-risk children and 11 very low-risk children (3%; p=0.86). ISS-related 'pathological' MR accounted for 22 of 25 (88%) WHF 'borderline RHD' cases.

Conclusions

ISS are a ubiquitous finding amongst South African schoolchildren from all risk profiles and are regularly identified as the underlying mechanism of WHF 'pathological' MR in 'borderline RHD' cases. A detailed MV assessment with an emphasis on ascertaining the underlying mechanism of dysfunction could reduce the reported numbers of screened cases misclassified as 'borderline RHD'.

3.2. Introduction

The finding of mitral regurgitation (MR) is a critical discriminator in echocardiographic rheumatic heart disease (RHD) screening and should prompt a detailed search for morphological features of RHD. The current 2012 World Heart Federation (WHF) criteria for the diagnosis of RHD use a Doppler-based system to grade regurgitation and differentiate between so-called 'physiological'- and 'pathological' MR (Table 1.

1.The abridged World Heart Federation diagnostic screening criteria for rheumatic heart disease.)[1] The WHF criteria further categorise screened subjects with 'pathological' MR into those with 'definite RHD' (at least two additional morphological features) and a 'borderline RHD' category in which 'pathological' MR is sufficient as a sole criterion. Although isolated 'pathological' MR remains non-specific as an indicator of RHD, the underlying premise has been that 'pathological' MR identified in high-risk children (in the absence of an identifiable pathology), is likely to represent rheumatic involvement. [1,2]

Nevertheless, there is consensus that the borderline group represents a diverse spectrum that includes RHD, but owing to a reduction in diagnostic specificity may equally well contain cases of alternate 'pathologies', including variants considered on the 'upper limit of normal'.[2,3] This is of concern, mainly as borderline cases with isolated WHF 'pathological' MR constitute between 32%-92.3% of reported cases with WHF 'screen-positive' disease.[4–10,11]A current research priority in RHD screening is to define echocardiographic features that can better delineate the presence of true RHD and reduce the size of the borderline group. A logical approach to differentiate normal from abnormal cases in this category would be to address the WHF method of MR evaluation. The Doppler-based system, while useful in standardising the classification of mild MR, remains a non-specific assessment and does not offer additional diagnostic clarity as to the underlying aetiology of dysfunction. A mechanistic evaluation of MR built on established international guidelines and standards may allow for further differentiation within the borderline group.[12]

Under the auspices of the Echo in Africa program (EIA), we have identified a common unifying mechanism of MR in a proportion of screened high-risk children with WHF 'pathological' MR The MR originates from slit-like separations (inter scallop separation- ISS) between the scallops of the PMVL and the MR is seen to move vertically down through the PMVL, rather than across the line of valvular coaptation. ISS is a ubiquitous finding throughout our high-risk cohort, and its association with MR has raised the question as to whether a possible non-rheumatic entity may be responsible.

This study aimed to establish the prevalence of ISS and ISS-related MR amongst a cohort of South African schoolchildren with high-and low-RHD risk. Furthermore, we define a mechanistic approach for MR evaluation in RHD screening that may assist in the differentiation between true RHD and the spectrum of normalcy.

3.3. Methods

Study design, setting, and participants

A prospective cross-sectional echocardiographic study was conducted. The high-risk cohort incorporated all EIA-screening data from a public, 'non-fee' paying secondary school situated in Khayelitsha, a large informal township located on the outskirts of the Cape Town Metropole. According to standardised South African measures of socioeconomic disadvantage, the Khayelitsha household income is considerably lower than the national average with a significant proportion(> 20%) of the community living below the 'poverty line'.[13] The very low-risk cohort comprised all screening data from a private, independent secondary school situated in the Cape Winelands. An a priori hypothesis assumed that the RHD risk profile of attending scholars (i.e. risk of

poverty, overcrowded households and poor access to adequate healthcare) was low. This hypothesis was supported by the school's annual tuition fee of R130 000, which put into context, is more than the total annual income in over 25% of Khayelitsha's households.[14]

Screening procedure

Study participants from the very low-risk cohort were enrolled between March and April 2018 and the high-risk cohort in October 2018. All schoolchildren (aged 13-18) with valid consent were screened in a tailored examination room at their respective schools. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the University of Stellenbosch's Health Sciences Ethics committee (N14/04/038 & S17/02/030) and the Western Cape Department of Education.

The initial screening echocardiogram was captured using a portable handheld (HH) device (GE Vscan version 1.2, Milwaukee, USA). Screened cases with MR ≥ 1.5cm qualified for a comprehensive echocardiographic study. This study was performed during the same sitting using a laptop machine (GE Vivid I, Milwaukee, USA). Both studies followed a standardised protocol which included a detailed analysis of the MV in long-and short-axis including a parasternal sweep (scanning method of the MV and associated MR incorporated into our RHD screening protocol).[15,16] All screening and comprehensive echocardiograms in the very low-risk cohort were performed by the principal investigator(LDH). All screening studies in the high-risk cohort were initially captured by a team of eight British Society of Echocardiography (BSE)-accredited sonographers, under the supervision of experienced RHD screeners. (LDH, GWL). All screening studies were deidentified and uploaded to an EchoPAC™ database for subsequent off-line analysis. Standardised echocardiographic settings were utilised according to the WHF guideline and those described in handheld-based screening studies.[1,11]

Echocardiographic definitions

ISS

ISS are defined as slit-like separations in the PMVL, identified in the parasternal short-axis view (PSSAX) while sectioning the leaflet at the tips (Media clip 3. 1). ISS are typically seen to fold open during diastole and close during systole. The PMVL has been arbitrarily divided into sections (P1, P1/P2, P2, P2/P3, P3) to assist the reviewer in approximating the location of the ISS (Figure 3. 1).

RHD

RHD classification was based on the WHF diagnostic criteria for RHD (see Table 1. 1). The criteria classify RHD as either 'definite' or 'borderline' according to a combination of WHF 'pathological' valvular regurgitation and morphological features of RHD.

Mechanistic evaluation of MR in RHD screening

A Carpentier-style[17] classification of mitral valve regurgitation was used to identify the following mechanisms relevant to our screening population:

Normal leaflet motion

Leaflets with normal motion were categorised into those with an MR mechanism attributable to an underlying ISS (MR originating from slit-like separations between the scallops of the PMVL) or a cleft involving the anterior mitral valve leaflet (AMVL). The origin of an ISS-related MR jet is confirmed on an optimised parasternal short-axis (PSSAX) view (ensuring to section the tips of the mitral valve leaflet). Typically, the MR jet is appreciated at, or immediately adjacent to the ISS as a fixed spot of colour or seen to be moving in a vertical up-down fashion through the PMVL rather than across the line of valvular coaptation during systole (Media clip 3. 2). The MR jet morphology must be scrutinised in an orthogonal plane (PSLAX) to exclude the possible differential of a posteriorly directed jet associated with PMVL restriction or AMVL prolapse.

Excessive leaflet motion

Leaflets with excessive motion were further categorised into cases with either mitral valve prolapse (MVP) or MVP-spectrum. MVP was diagnosed when the leaflet was seen to move beyond the annular plane (>2mm) in a long axis orientation, in keeping with current consensus guidelines.[12] MVP-spectrum was diagnosed in cases where some portion of the leaflet was seen to move beyond the annular plane with associated tip malcoaptation. In these cases, there was no associated PMVL restriction, nor was the valve seen to prolapse >2mm beyond the annular plane in a long axis orientation. Typically, the MR jet is seen to emanate across the line of valvular coaptation, exhibiting a broader colour Doppler jet on the optimised PSSAX view than typically seen for MR through an ISS (

Media clip 3. 3 and Media clip 3. 4).

Restricted leaflet motion

Systolic and diastolic restriction of the PMVL with resultant malcoaptation of the PMVL and AMVL during systole gives the impression of AMVL' tip prolapse' or 'excessive leaflet motion'. These terms are synonymous and generate so-called 'pseudoprolapse' of the AMVL which cannot be regarded as true prolapse, as the AMVL is seen to be in its normal position at end-systole and does not cross the annular plane.[16] 'Pseudoprolapse of the AMVL generates the characteristic posteriorly directed jet of rheumatic MR with a similar broad Doppler jet exhibited on the optimised PSSAX view (Media clip 3. 5, Media clip 3. 6, Media clip 3. 7). Restricted PMVL motion primarily during systole ('tethering') has a wide differential and includes any aetiology known to alter the geometry of the left ventricle. This category is not likely to be encountered during screening amongst asymptomatic children.

Indeterminate

Screened cases with MR whose underlying mechanism was not discernible were classified as 'indeterminate'.

Data analysis

The initial screening and relevant comprehensive studies of both the high-and very low-risk cohort were evaluated by the lead investigator (LDH). ISS were identified from the initial study, and only discernible cases were included in the overall count. Only the comprehensive scans of handheld studies with positive findings (MR ≥1.5cm) were reviewed and were classified according to the current WHF criteria. All screening and

related comprehensive studies identified with MR were categorised according to the classification system previously described.

Statistical analysis

Deidentified data were analysed using Stata (version 12, Stata Corp, Texas, USA). Categorical variables were compared using the $\chi 2$ or Fisher's exact test where appropriate. A 2-sided P value < 0.05 was considered statistically significant. For qualitative variables, proportions along with 95% confidence intervals were calculated. Cohen's kappa statistic was used to evaluate the inter-rater agreement between the lead investigator and a second, blinded reader who was uninvolved in the initial screening (AJK). Due to the relatively small sample size, a reread of all comprehensive studies whose initial HH screening study had an MR jet ≥1.5cm was performed. The lead investigator and the reader were required to note the presence of an ISS and whether the mechanism of MR was attributable to an ISS using the provided definitions. The interpretation of kappa values was based on the Landis and Koch guidelines.[18] The proportion of agreement was reported as mean percentages with a 95% CI for inter-rater agreement.

Sample size

According to an autopsy series, ISS are present in virtually all healthy human hearts.[19] The frequency of echocardiographic detection is unknown. As this is the first comprehensive echocardiographic study of ISS, we set our anticipated frequency of detection at 50% amongst a population size of 1,000,000. Accordingly, we determined that a sample size of 384 subjects from each cohort would accurately determine the rate of ISS between high-and very low-risk RHD populations, with a 95% confidence interval (CI). The actual enrolled sample size of the very low-risk cohort (359 participants) was determined to have a minimal impact on the power of the study.

3.4. Results

Cohort demographics

A total of 759 children were enrolled for study participation. Our screening cohort comprised of three population groups- black-, white- and mixed South African (an ethnic group of Khoisan-European-African-Malay mixed ancestry). The demographic characteristics of 400 children at high-and 359 children at very low-risk are presented in Table 3. 1. Both cohorts had a mean age of 15.5 years. The population group of the high-risk cohort was exclusively black South African($p<0.01$) and was predominantly female (68%; $p<0.01$). This is in contrast to the very low-risk cohort, which was predominantly white South African (89%; $p<0.01$), with a more balanced sex distribution (52.6% female).

WHF RHD assessment

No very low-risk child met WHF criteria for 'definite RHD' compared with two children in the high-risk cohort (prevalence, 5 per 1000 [95% CI, 1.4-18.0]; p=0.5). 14 very low-risk children (prevalence, 39 per 1000 [95% CI 23.4-64.4]) and 11 high-risk children (prevalence, 27.5 per 1000 [95% CI 15.4-48.6]) met criteria for 'borderline RHD' (odds ratio, 0.69 [95% CI, 0.31-1.55]; p=0.37). The prevalence of RHD ('borderline' and 'definite') in the high-risk cohort was 32.5 cases per 1000 (95% CI, 19.1-54.8). The odds ratio for a diagnosis of 'definite- 'or 'borderline RHD' in the high-risk cohort compared to the very low-risk cohort was 0.82[95% CI, 0.38-1.78]; p=0.62).

Prevalence of ISS and ISS-related MR

A discernable ISS was identified in 278 (69.5%, [95% CI 64.8%-73.8%]) cases from the high-risk cohort and in 269 (74.9%, [95% CI 70.2%-79.1%]) cases from the very low-risk cohort(p=0.10;Table 3. 2) A single ISS predominated in both cohorts with 216 cases (77.7%, [95% CI 72.4%-82.1%]) in the high-risk- and 225 cases in the very low-risk cohort (62.6%, [95% CI 57.5%-67.5%] p<0.0001). Isolated ISS were most frequently identified in the P2/P3 position, constituting the majority of ISS cases in both the high-risk (n=142; 51%, [95% CI 45.2%-56.9%]) and very low-risk cohort (n=136; 50.5%, [95% CI 44.6%-56.4%]; p=0.93). MR was detected in 100 cases in the high-risk cohort (25%; 95% CI, 21-29.5) and 103 cases in the very low-risk cohort (28.7%, 95% CI, 24.26-33.58; p=0.25; Table 3. 3). Overall, 547 cases were identified with ISS (72%), 104 cases of these cases had any ISS-related MR (13.7%), but only 22 cases (4%) demonstrated WHF 'pathological' MR (Table 3. 3). After comprehensive echocardiography, an underlying ISS was identified as the underlying mechanism of isolated WHF 'pathological' MR in 11 (2.8%) high-risk children and 11 very low-risk children (3%; p=0.86; Table 3. 3, Table 3. 4). In total, ISS-related MR cases comprised 22 of the 25 WHF 'borderline RHD' cases or 88% of the total borderline group (Table 3. 3). There were no additional morphological or mechanistic features of RHD in these cases.

Mechanistic evaluation of MR

Handheld screening determined the underlying mechanism of MR in 56(56%) high-risk MR cases and 56(54.4%) very low-risk cases(p=0.88; Table 3. 4) Of these cases, ISS-related MR was identified in 49 high-risk children (49.4%) and 55 (53.3%) very low-risk children (p=0.67). There were no cases of MVP identified in the high-risk cohort compared to a single case in the very low-risk cohort (prevalence, 2.8 per 1000 [95% CI, 0.5-15.6], p=0.47). There were 5 cases of MVP-spectrum identified in the high-risk cohort (prevalence, 12.5 per 1000 [95% CI, 5.4-28.9]) and none in the very low-risk cohort (p=0.06). 'Pseudoprolapse' of the AMVL was identified in both cases of WHF 'definite RHD'. The MR mechanism on handheld screening was indeterminate in 44(44.4%) high-risk children and 47(45.6%) very low-risk children(p=0.39). Of the 27 WHF 'pathological' MR cases, only three cases (11.1%) had an indeterminate mechanism of MR (Table 3. 4).

Assessment of Interobserver Agreement

The agreement between readers on the presence of an ISS was substantial (kappa=0.60; 0.46-0.74) with a proportion of agreement of 88.9%. There was almost perfect agreement between readers on whether the mechanism of MR was attributable to an ISS (kappa=0.90;0.84-0.96) with a proportion of agreement of 96.3%

3.5. Discussion

This is the first descriptive study of the echocardiographic prevalence of ISS of the PMVL. Our findings suggest that ISS are a ubiquitous entity amongst South African children and are frequently identified as the underlying mechanism of WHF 'pathological' MR in children, irrespective of RHD risk. An echocardiographic assessment that incorporates a mechanistic evaluation of MR may prevent misclassification of RHD in a large proportion of screened children.

The present study has three main findings. Firstly, we present a novel, reproducible screening definition of ISS that is synonymous with previous accounts of PMVL' slits', 'splits' and 'indentations'; a normal variant of the PMVL.[13,22-25] ISS were a ubiquitous finding in our study and were identified in over two-thirds of participants in both screened cohorts (Table 3. 3). Furthermore, we present novel data suggesting that the PMVL appears to have characteristic patterns of ISS involvement. The majority of observed ISS were isolated and located in the medial aspect of the PMVL(P2/P3), accounting for over 50% of all isolated ISS in both cohorts (Table 3. 2).

Secondly, we have introduced a mechanistic evaluation to assess and define the mechanism of MR in RHD screening. In doing so, we reproducibly identified ISS-related MR as the prominent mechanism of MR in the majority of cases, including those designated with WHF 'pathological' MR (Table 3. 3).
It is important to remember that the majority of MR cases, even if designated WHF 'pathological' MR, constituted clinical mild or very mild MR. From our experience, the exact mechanism of MR was more challenging to ascertain in those cases with the very mildest MR as the valve morphology and motion approximates normality to a high degree in these cases. Although only 55.2% of all MR cases could be allocated to a clear mechanistic group, this increased to 88.8% when considering only the WHF 'pathological' MR group (Table 3. 4).

Although the rate of WHF 'pathological' MR was low amongst all ISS-related MR cases (2.9%), these cases constituted the bulk of the WHF 'borderline RHD' group (Table 3. 3). ISS was identified as the mechanism in 11 high-risk (2.8%) and 11 (3%) low -risk children (p=0.86) with no additional morphological features of RHD included into the borderline group, constituting 22 out of the 25 cases (88%) with borderline disease. For the first time, this finding of a common, non-rheumatic mechanism for WHF' pathologic' MR (ISS) challenges the dogma that WHF 'pathological' MR necessarily points to RHD in high-risk communities. The adoption of a mechanistic evaluation of MR in RHD screening presents a critical opportunity to address the 'borderline' conundrum by significantly reducing the size of the borderline RHD group in large-scale screening studies.

The third finding of interest in this study relates to the prevalence of WHF 'borderline RHD' identified in the very low-risk cohort. While the low prevalence (0 cases) of WHF 'definite RHD' supports this particular school's a priori allocation of low risk, we did, however, identify 14 cases of WHF 'pathological' MR, none of which demonstrated concomitant morphological features of RHD. Our figures are appreciably higher than the 1.3% (95% CI, 0.6%-2.9%) reported by Webb et al[2] and the 0.2% (95% CI, 0.05%-0.69%) by Roberts et al[4] in their respective low-risk New-Zealand and Australian cohorts. To our knowledge, this is the first published RHD screening study of very low-risk children in Southern Africa and consequently have no regional data with

which to compare our findings. We can only speculate as to the potential factors that may have contributed to our findings. It is possible that alternative population groups from different areas in the world may exhibit diverse MV characteristics that either predispose them to more (or less) MR. For instance, these characteristics could include variations in the number, location, and size of ISS. Our EIA experience supervising volunteer BSE-accredited sonographers has highlighted an essential human factor that deserves consideration. Despite our volunteer's training, there is an associated learning curve to adequately identify mild forms of MR and in particular, capturing a complete MR CW envelope. We postulate that our unprecedented findings of borderline disease may have been in part, a function of a high quality, detailed echocardiographic study with strict adherence to the WHF criteria.

Inter-scallop separation a cleft of the PMVL?

The current WHF criteria stipulate that congenital causes of 'pathological' MR (such as cleft MV) should be excluded before further analysis to avoid misidentification as RHD.[1]

Currently, there is no consensus on the echocardiographic definition of a PMVL cleft. Some authors define clefts by their projection into the PMVL and use an arbitrary cut-off of a depth of more than 50% of the adjacent scallop[21,23]. Some require that the definition only includes clefts that extend to the annulus and are associated with some degree of regurgitation[22]. Other interpretations necessitate the presence of concomitant cardiac anomalies[19], while some simply define clefts as defects located between the 'normal' inter-scallop position.[23,24] In their autopsy series of normal hearts, Victor et al. established that ISS morphology (size and number) appear to be unique to each heart [19] This finding calls into question whether instances of larger ISS (i.e. >50% of the PMVL) should merit an exclusive status as a cleft, having been identified as part of the normal spectrum.

While ISS has been key to the identification of the underlying mechanism of MR in a large proportion of our cases, we found the systematic assessment of ISS size using 2D echocardiography both technically challenging and imprecise. Furthermore, in our experience, there appears to be no predictable association between the anatomical size of the ISS and the degree of functional deficit. This would argue against the creation of an arbitrary definition of a PMVL' cleft' as it does not inform the screening process.

Therefore, in the screening context, it makes sense to refer to AMVL clefts as 'clefts', but an ISS should be dealt with as a normal variant of the PMVL with relevance in RHD screening. Nevertheless, given the ubiquitous nature of ISS, it is plausible that subjects with an identifiable ISS with associated MR could have concurrent true morphological features of RHD. Consequently, we would not advocate labelling a case with ISS-related MR as 'congenital', before a detailed assessment for morphological features of RHD.

Limitations

There were notable differences between the two selected cohorts of children. The population group reflected in the high-risk cohort was exclusively Black South African. This finding echoes a reality in South Africa, where the majority of Black South Africans (64.2%) continue to live below the 'poverty line' as compared to only 1% of White South Africans.[13]

The sex ratio in the high-risk cohort was predominantly female, in part reflecting a documented trend of high drop-out rate amongst males attending South African secondary schools in low socioeconomic communities.[25]

The size of our cohort and by implication, the relatively low prevalence of MR, limits the generalizability of our findings. Further definitive study is required to address outstanding questions that include the prevalence of ISS-related MR in larger high-risk populations, the long-term prognosis of ISS-related MR and the possibility of variability amongst different population groups.

The prevalence of WHF 'pathological' ISS-related MR was not different in the high- and the very low-risk cohorts and supports the hypothesis that coexisting RHD is not a requirement for the development of WHF 'pathological' MR through an ISS (Table 3. 4). However, because of the known modifying effect that ISS can have on the severity of MR in both functional and degenerative valve disease,[21,26] further study is required to evaluate the impact that coexisting RHD may have on the severity of the ISS-related MR to avoid underdiagnosis of RHD in cases with coexisting RHD and prominent ISS.

3.6. Conclusion

Inter-scallop separations of the posterior mitral valve leaflet are a ubiquitous finding amongst South African schoolchildren from all risk profiles and are regularly identified as the underlying mechanism of WHF 'pathological' MR in 'borderline RHD' cases. A detailed mitral valve assessment with an emphasis on ascertaining the underlying mechanism of dysfunction could reduce the reported numbers of screened cases misclassified as borderline disease.

3.7. References

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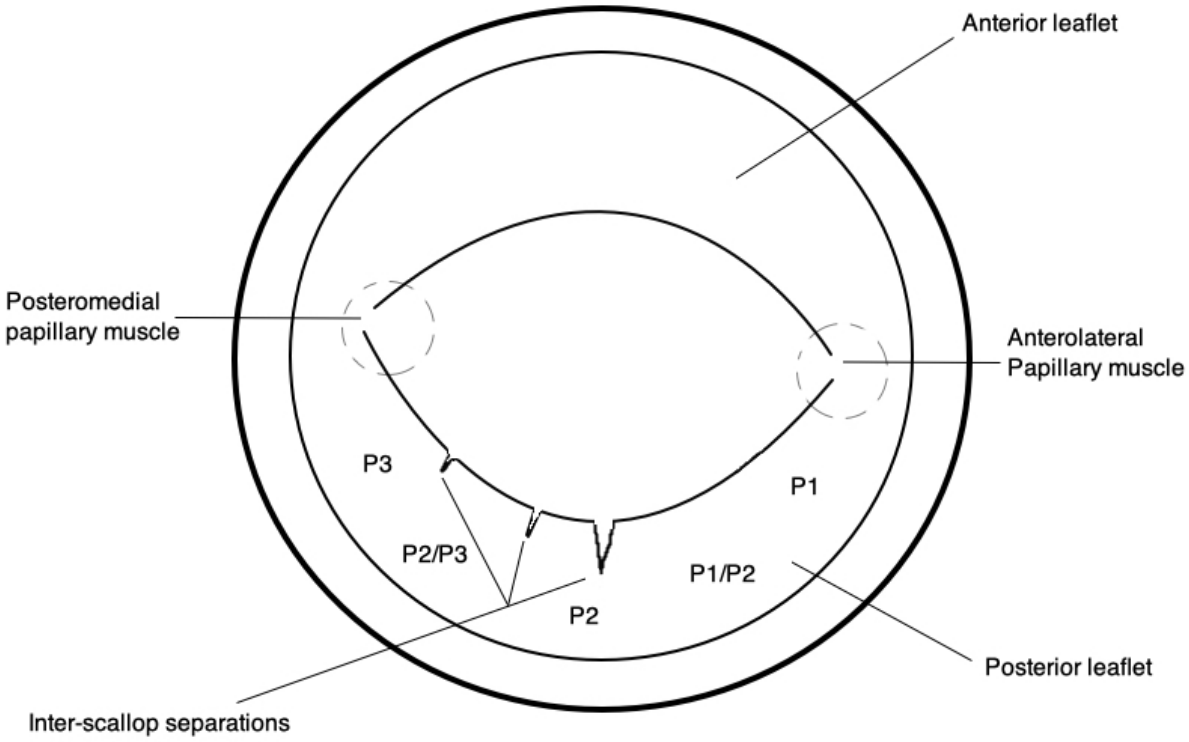
3.8. Figures

Figure 3. 1. Echocardiographic representation of a mitral valve in a parasternal short-axis view.



The posterior leaflet has been sectioned at the tips demonstrating three examples of inter-scallop separations (ISS) of variable location, size and shape.

3.9. Tables

Table 3. 1. Demographic parameters of the high-and very low-risk cohorts

	Very low-risk cohort (n=359)		High-risk cohort (n=400)	
	n	**%**	**n**	**%**
Gender (% female)	189	52.6	272*	68
Age (mean)	15.5		15.5	
Population group				
Black South African	17	4.7	400*	100
White South African	319*	88.8	0	0
Mixed South African	23*	6.4	0	0

*P <0.001

Table 3. 2. Frequency and location of inter-scallop separation(s) detected by handheld echocardiography

	Low risk cohort (n=359)		High risk cohort (n=400)	
	n	**%**	**n**	**%**
No/indeterminate ISS*	90	25	121	30.2
Isolated ISS†				
P1	1	0.4	8§	2.9
P2	40	14.8	37	13.3
P3	42	15.6	16§	5.8
P1/P2	6	2.2	13	4.7
P2/P3	136	50.6	142	51
>1 ISS†				
Double ISS	37	13.8	55	19.8
Treble ISS	7	2.6	6	2.2
Quadruple ISS	0	0	1	0.3
Total	**269**	**100**	**278**	**100**

* Calculated as a percentage of the entire cohort

†Calculated as a percentage of cases with identifiable inter-scallop separation of the PMVL

§ P<0.05)

Table 3. 3. Amalgamated study data cross referencing MR prevalence, ISS and WHF 'screen-positive' disease

	Very low-risk cohort (n=359)	High-risk cohort (n=400)	Total(n/%)† (n=759)
All ISS cases(n/%)*	269(74.9)	278(69.5)	547(72)
ISS-related MR (n/%)*	55(15.3)	49(12.3)	104(13.7)
ISS-related 'pathological' MR (n/%)*	11(3)	11(2.8)	22(2.9)
All cases with any MR (n/%)*	103(28.7)	100(25)	203(26.7)
MR screening ≥1.5cm (n/%)*	19(5.3)	23(5.8)	42(5.5)
WHF 'pathological' MR (n/%) *	14(3.9)	13(0.25)	27(3.6)
WHF 'screen-positive' cases (n/%)*	14(3.9)	13(3.3)	27(3.6)
WHF 'borderline RHD' (n/%) *	14(3.9)	11(2.8)	25(3.3)
WHF 'definite RHD' (n/%) *	0(0)	2(0.5)	2(0.3)

* Calculated as a percentage of the respective cohort

† Calculated as a percentage of the total enrolled participants

MR, mitral regurgitation; ISS, inter-scallop separation; WHF, World Heart Federation; RHD, rheumatic heart disease

Table 3. 4. Degree and associated mechanism of MR amongst screened low- and high-risk children

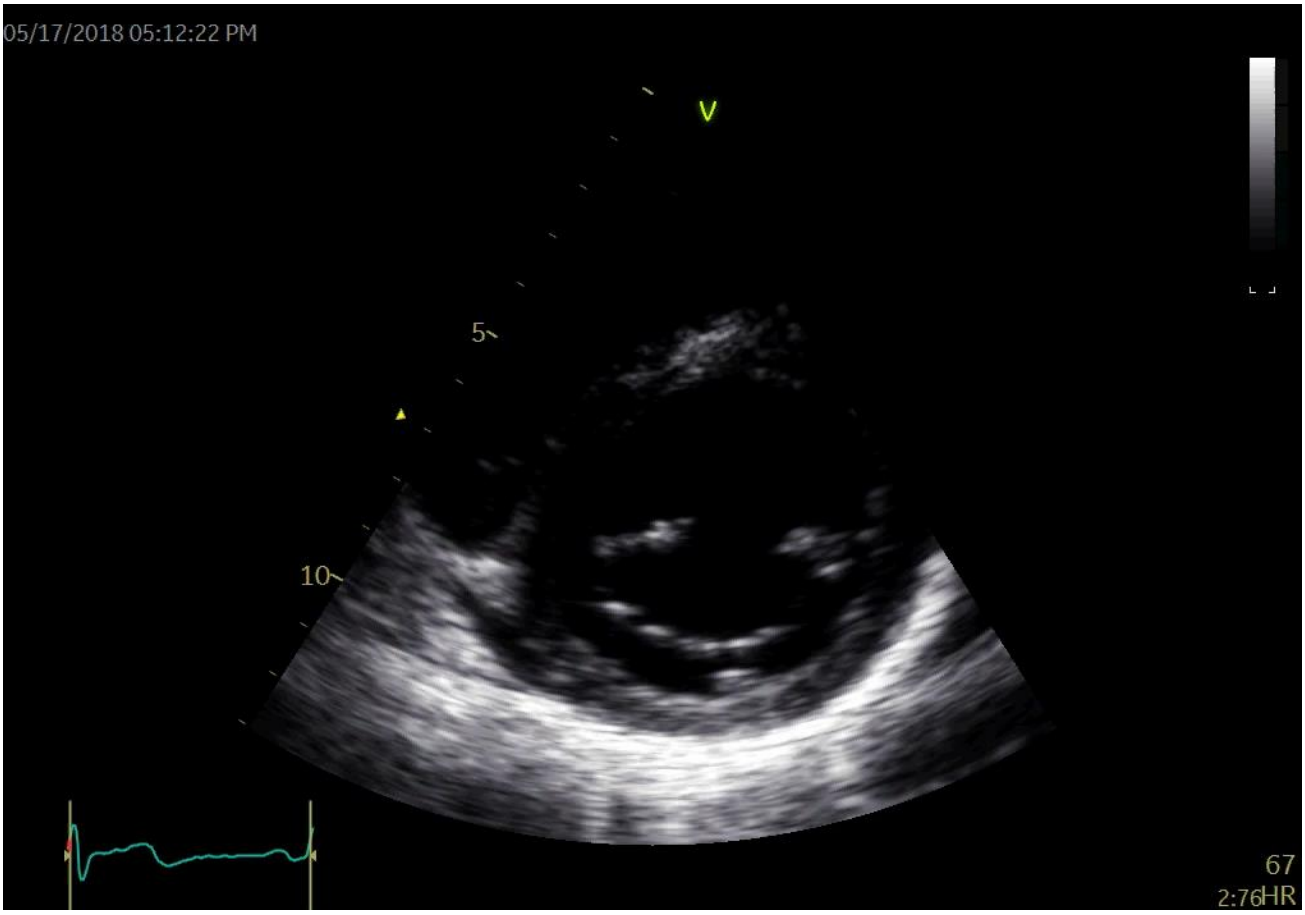
	Low- risk cohort (n=359)					High-risk cohort (n=400)				
	Mechanism of MR					Mechanism of MR				
MR grade	ISS (n/%)	MVP/MVP-spectrum (n/%)	Pseudoprolapse (n/%)	Indeterminate (n/%)	Total* (n/%)	ISS (n/%)	MVP/MVP-spectrum (n/%)	Pseudoprolapse (n/%)	Indeterminate (n/%)	Total* (n/%)
MR (All)†	55(53.4)	1 (1)	0(0)	47(45.6)	103(28.7)	49(49)	5(5)	2(2)	44(44)	100(25)
MR (1cm <2cm) †	34(50)	1(1.5)	0(0)	33(48.5)	68(18.9)	28(49.1)	3(5.3)	0(0)	26(45.6)	57 (14.3)
MR (≥ 2cm) †	15(65.2)	0(0)	0(0)	8(34.8)	23(6.4)	12(60)	2(10)	2(10)	4(20)	20(5)
MR (WHF path) †	11(78.5)	0(0)	0(0)	3(21.4)	14(3.9)	11(84.6)	0(0)	2(15.4)	0(0)	13(3.3)

*Calculated as a percentage of the entire cohort (MR, mitral regurgitation; ISS, inter-scallop separation; MVP, mitral valve prolapses; WHF path, World Heart Federation 'pathological' MR on comprehensive study)

†Calculated as a percentage of the total in each specific category

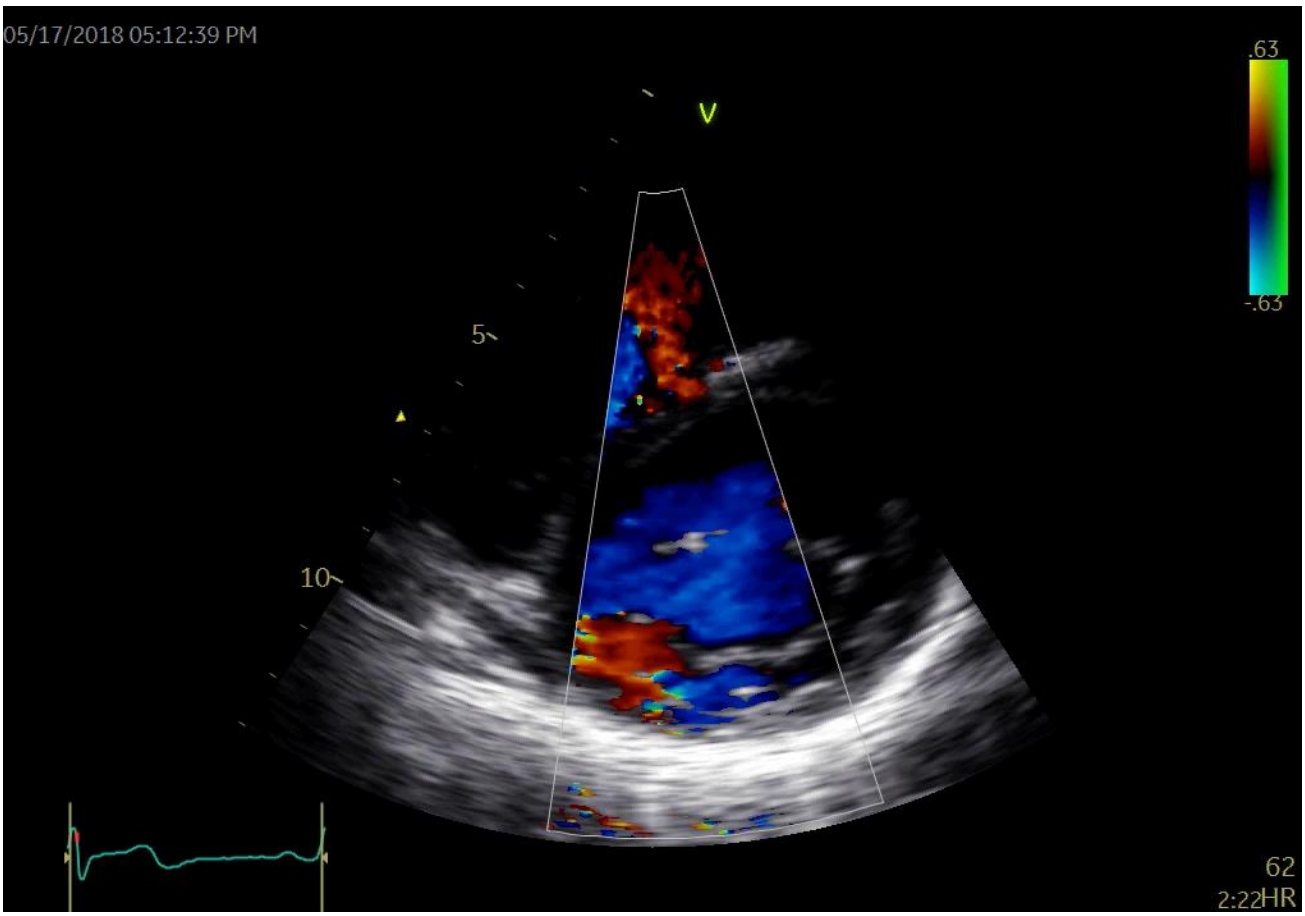
3.10. Media clips

Media clip 3. 1. Parasternal short-axis view of a normal mitral valve with multiple inter-scallop separations of the posterior mitral valve leaflet



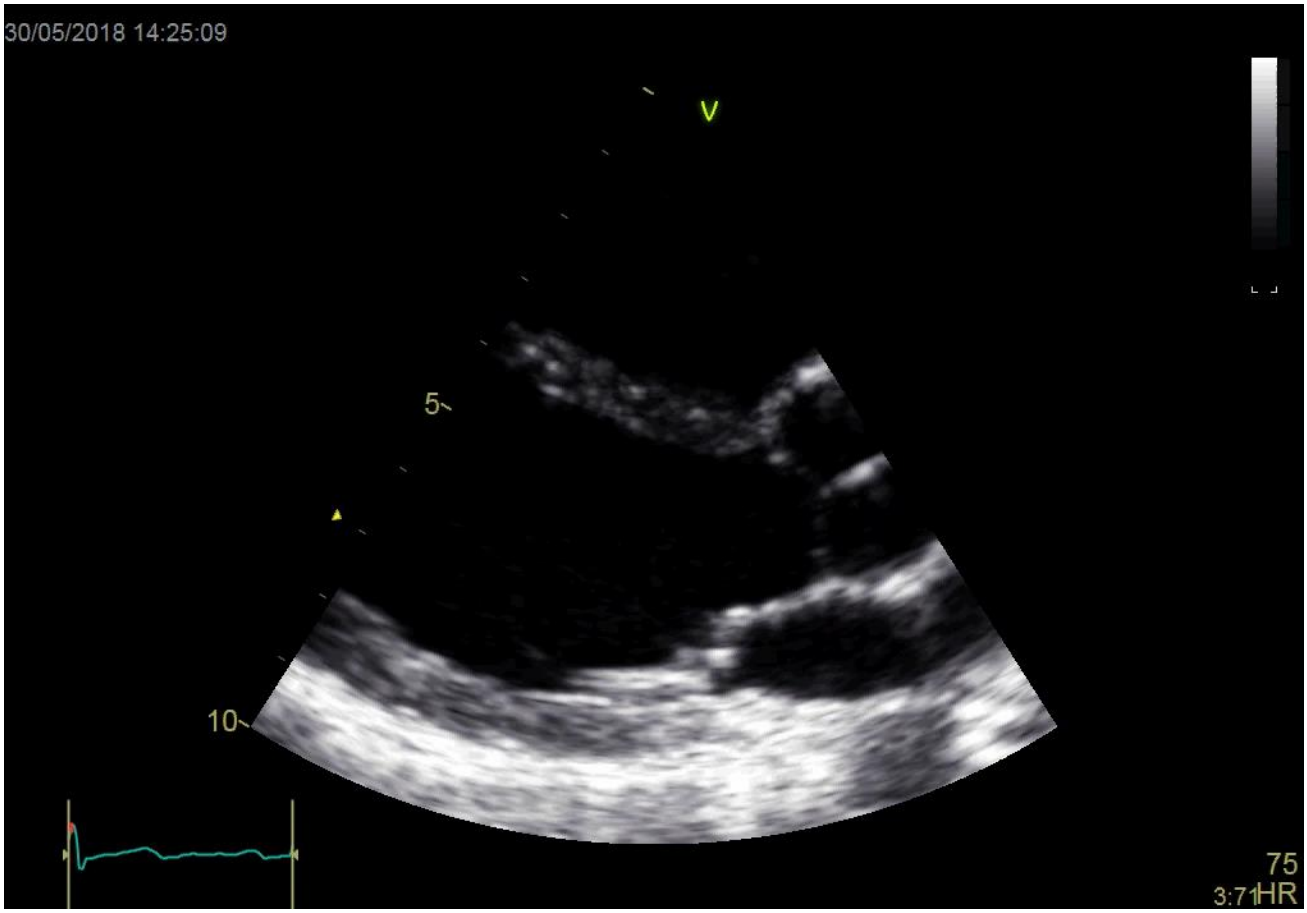
Care is taken to section the leaflet at the tips: multiple inter-scallop separations (ISS) are visible and are located in the P1, P2, P2/P3 and P3 portions of the posterior mitral valve leaflet (PMVL).

Media clip 3. 2. Parasternal short-axis view with focused colour Doppler over the mitral valve of the case presented in Media clip 3.1.



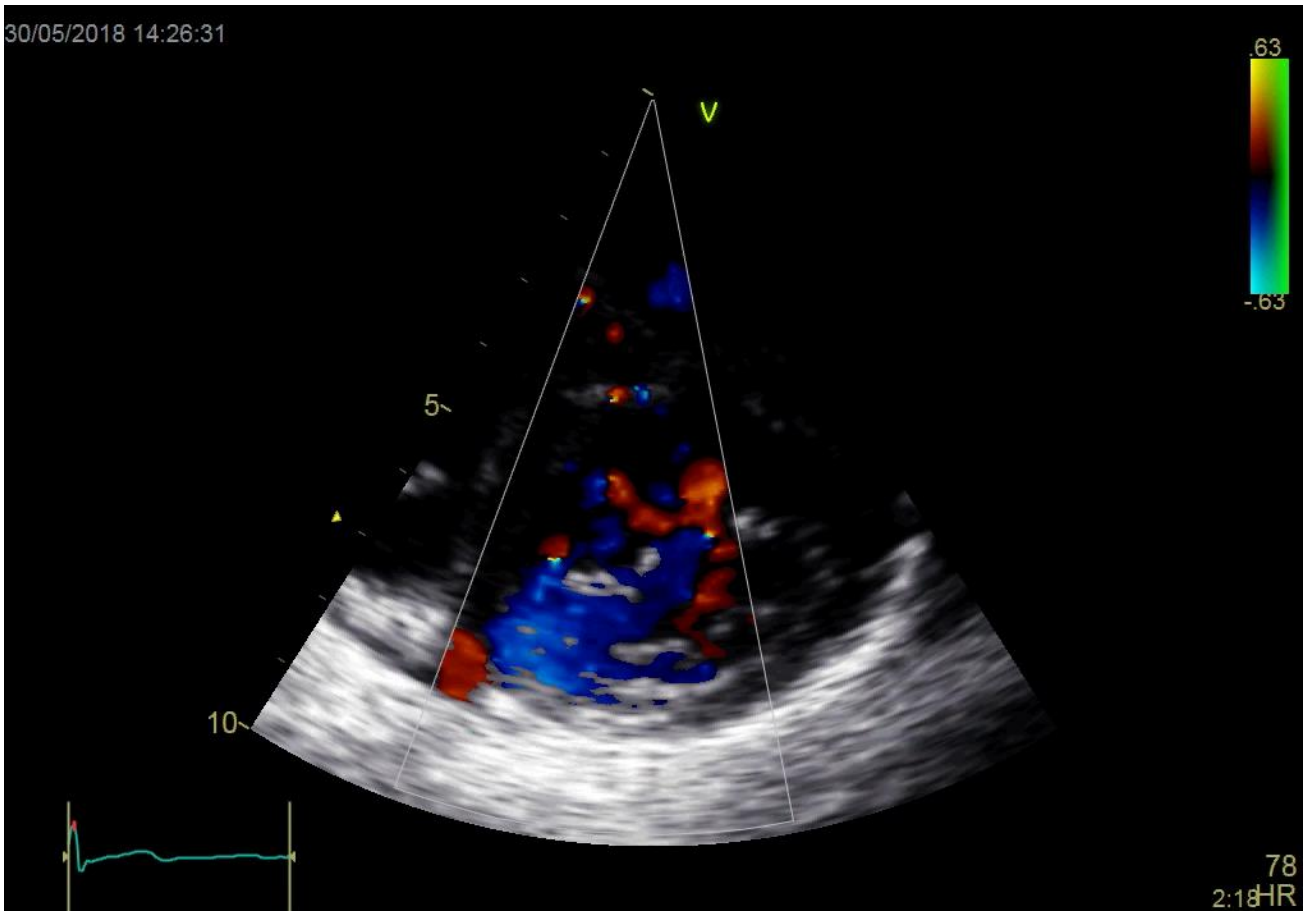
Colour Doppler over the mitral valve (MV) confirms a P2/P3 ISS as the underlying cause of the MR

Media clip 3. 3. Parasternal long-axis view of a screened case with mitral valve prolapse spectrum



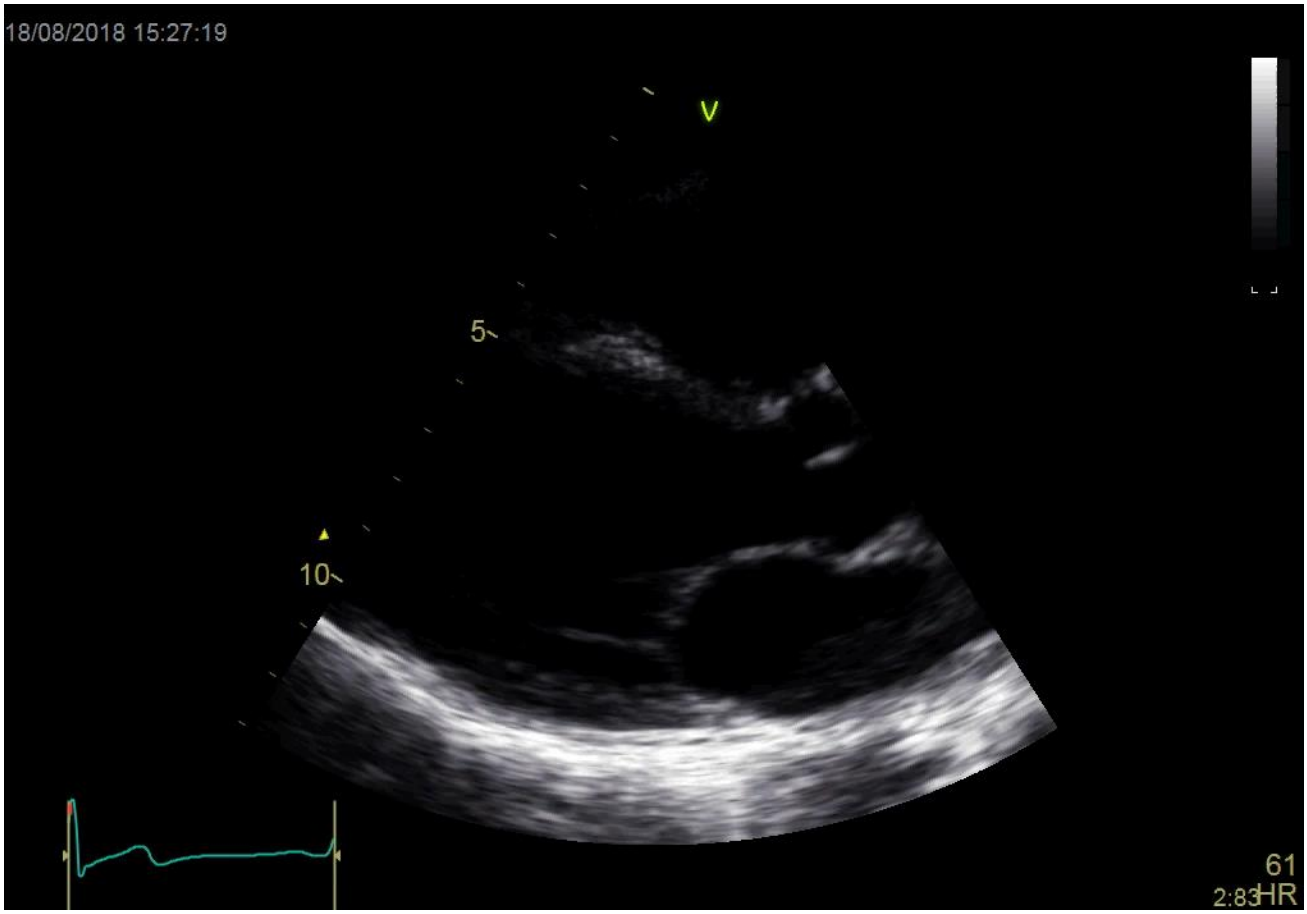
There is no associated PMVL restriction, nor is the valve seen to prolapse >2mm beyond the annular plane in a long axis orientation.

Media clip 3. 4. Parasternal short-axis view with focused colour Doppler over the mitral valve presented in Media clip 3.3.



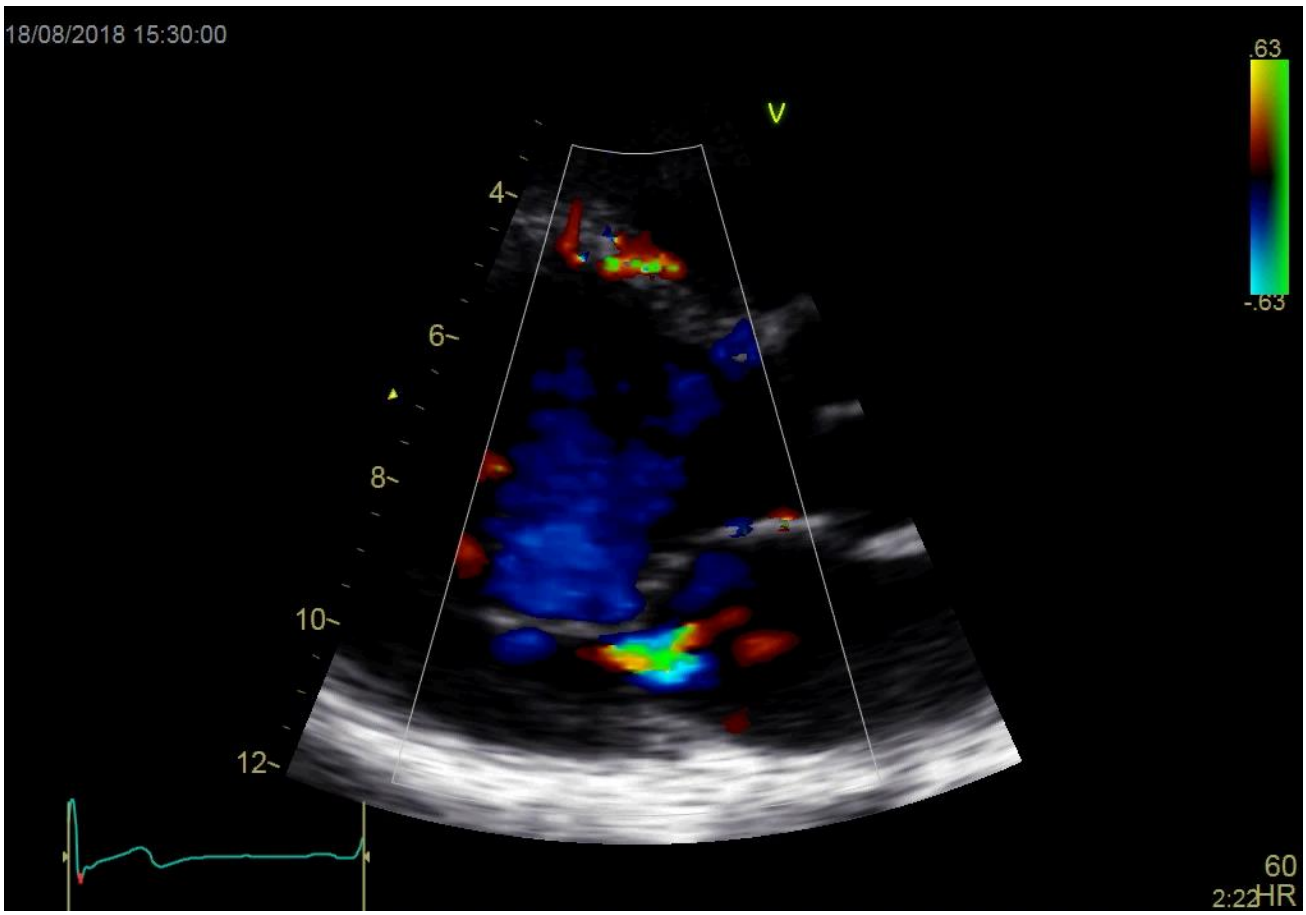
The MR jet is seen to emanate across the line of valvular coaptation, exhibiting a broad colour Doppler jet.

Media clip 3. 5. Parasternal long-axis view of a rheumatic mitral valve



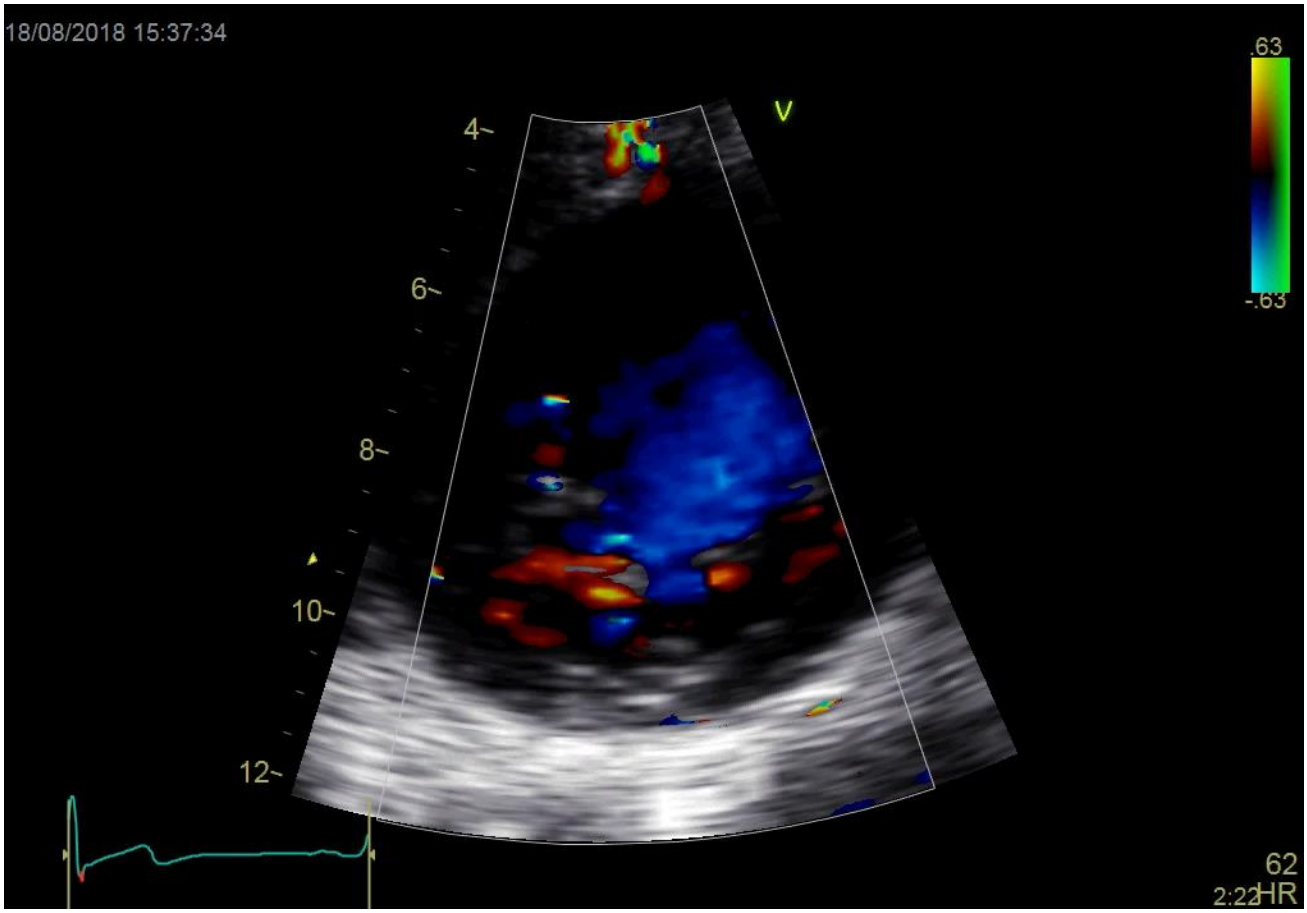
There is suggestive rheumatic-related restriction of the anterior mitral valve leaflet (AMVL) and PMVL. The A2 segment of the AMVL is seen to 'prolapse' past the P2 segment of the PMVL. This mechanism is more correctly termed 'pseudoprolapse' as the AMVL is in its normal position at end systole. The impression of A2 prolapse is related to PMVL systolic restriction with resultant malcoaptation of the PMVL and AMVL during systole, generating the characteristic posteriorly directed jet of rheumatic MR.

Media clip 3. 6. Parasternal long-axis view with focused colour Doppler over the mitral valve of the case presented in Media clip 3.5.



This clip demonstrates the characteristic posteriorly directed MR jet encountered in chronic rheumatic MR

Media clip 3. 7. Parasternal short-axis view with focused colour Doppler over the mitral valve of the case presented in Media clip 3.5.



The MR jet is seen to emanate across the line of valvular coaptation, exhibiting a broad colour Doppler jet similar to that seen in mitral valve prolapse and prolapse spectrum.

Chapter 4: Echocardiographic assessment of subclinical rheumatic heart disease: The Echo in Africa project

Chapter four is a submission-ready manuscript reporting the results from the first five years of the Echo in Africa project (large-scale RHD screening project in the Western Cape, South Africa). My role in the study included developing the study protocol and performing and capturing all echocardiographic assessments of all enrolled study participants. I am the primary author of the manuscript included in this chapter. CJ Lombard assisted with the statistical analysis of the study. AJK Pecoraro, MJ Monaghan and GW Lloyd reviewed the final manuscript. AF Doubell and PG Herbst were the co-supervisor and supervisor respectively. They supervised the study design and execution. Both reviewed the final draft of the manuscript.

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4.1. Abstract

Introduction

The World Heart Federation (WHF) criteria identify a large borderline rheumatic heart disease category that has hampered the implementation of population-based screening**.** The Echo in Africa (EIA) project aims to determine whether alternate, screening techniques could improve the specificity

of the current guideline. EIA has described a Carpentier-style mechanistic evaluation that may allow for further differentiation within the borderline group. The present study provides data from the first five years of the project.

Methods

A prospective cross-sectional study of underserved secondary schools in the Western Cape was conducted. Participants underwent a screening study with a handheld (HH) ultrasound device. Children with an abnormal HH study were re-evaluated with a portable laptop echocardiography machine. A mechanistic evaluation was applied in cases with isolated WHF 'pathological' MR (WHF 'borderline RHD').

Results

5255 participants (mean age 15 years) were screened. 3439 (65.8%) were female. 49 cases of WHF 'definite RHD' (9.1 cases/1000 [95% CI, 6.8-12.1 cases/1000]) and 104 cases of WHF 'borderline RHD' (19.5 cases/1000[95% CI,16.0-23.7 cases/1000]) were identified. A mechanistic evaluation identified ISS-related MR as the predominant underlying mechanism of MR in 48 cases of WHF 'borderline RHD' (46.1%).

Conclusion

Subclinical RHD remains a prevalent condition in the Western Cape. A novel mechanistic evaluation identified an ISS as the underlying mechanism of MR in a significant majority of borderline cases. Further study of this entity will inform the role of a mechanistic evaluation in reducing the size of the borderline disease group.

4.2. Introduction

Screening echocardiography is recognised as the diagnostic investigation of choice for the identification of rheumatic heart disease (RHD) amongst asymptomatic children.[1] The World Heart Federation (WHF) criteria were developed to standardise the reporting and diagnostic approach to 'subclinical' RHD (see Table 1. 1.The abridged World Heart Federation diagnostic screening criteria for rheumatic heart disease). The WHF criteria have reinvigorated RHD research in Sub-Saharan Africa[2-9] (

Figure 4. 1) and have galvanised amendments to official RHD health policy.[10] However, it remains unclear as to whether the criteria accurately identify those who would benefit from the early identification and institution of secondary prophylaxis. Unfortunately, the criteria create a large borderline group; a diagnostic category reserved for screened cases demonstrating some, but not all of the required criteria for a definite diagnosis. Longitudinal study of this heterogeneous cohort has yet to provide conclusive evidence that its identification and even treatment confers any prognostic benefit.[8,11-15] Consequently, there remains insufficient evidence to recommend widespread, population-based echocardiographic screening for RHD.[16,17] Since 2014, the Echo in Africa (EIA) project has provided echocardiographic RHD screening to high-risk school children in the Western Cape, South Africa. In addition to its primary humanitarian focus, the initiative was conceived with important research objectives: to critically appraise the WHF criteria and evaluate whether alternate, novel screening techniques could improve the specificity of the current WHF guideline. Recently, we have identified inter-scallop separations (ISS) of the posterior mitral valve (PMVL), a common, normal finding in healthy hearts irrespective of rheumatic risk.[18] This finding proved to be a prominent underlying mechanism of isolated WHF 'pathological' MR amongst screened South African schoolchildren with high- and very low-risk for RHD (see Chapter 3; Inter-scallop separations of the posterior mitral valve leaflet: a solution to the 'borderline RHD' conundrum?).

Here, ISS-related MR (without additional morphological features of RHD) accounted for up to 22 cases (88%) of borderline disease identified in our study. We propose that a mechanistic MR assessment would allow for further differentiation within the borderline category by identifying cases with alternative, non-rheumatic mechanisms of 'pathological' MR. The primary objective of this study was to present screening data from the first five years of the Echo in Africa project. The second objective was to assess the impact of a 'Carpentier-style' mechanistic evaluation amongst screened cases with WHF 'borderline RHD' (Subcategory B- isolated 'pathological' MR).

4.2. Methods

Study design and participants

EIA is a collaborative initiative between SUNHEART(a non-profit organisation established by the Division of Cardiology at Tygerberg Academic Hospital [TBH]) and the British Society of

Echocardiography (BSE). The EIA team conducted a prospective cross-sectional study in school children attending secondary state (public) schools in the Western Cape, South Africa. Ethical approval was obtained through the University of Stellenbosch and the Department of Education in the Western Cape, respectively (N14/04/038). The project's footprint spans three adjacent district municipalities, namely the City of Cape Town- (six schools), Drakenstein- (two schools) and Stellenbosch municipalities (two schools). Schools in low-income areas within each district municipality were selected based on a national quintile (NQ) score- a standardised poverty indicator that reflects the income, unemployment and level of education within each community.[19] Only schools classified by the Provincial Education Department as 'no-fee', national quintile (NQ) 1 and 2 were offered study participation. The study investigators approached the relevant governing bodies of each secondary school and offered study participation. Informed parental/guardian consent was required before study enrolment. Annual EIA screening camps, typically lasting four weeks were scheduled. Each week, roughly 10 BSE-accredited sonographers provided echocardiographic support for the project. All sonographers were required to complete a distance-learning module on rheumatic valve disease morphology and evaluation. After an initial hands-on training period, study investigators provided ongoing on-site tuition and support to all screeners during the program.

Echocardiographic evaluation

In 2014 and 2015, all enrolled study participants were screened in a purpose-renovated facility at Tygerberg Academic Hospital (TBH). All transthoracic echocardiogram (TTE) studies were performed by an adult cardiologist or a BSE-accredited sonographer under the guidance of an adult cardiologist. Participants were screened with a portable handheld device (HH; General Electric [GE™] V- scan) using a pre-defined study protocol (see supplementary material-Addendum A). This was followed by a comprehensive validation TTE study using a GE™ Vivid I portable laptop machine with a 2- to 3.6 MHz transducer probe (GE™ 3S). The validation study was performed according to the current BSE guideline for a standard adult TTE.[20] It was supplemented by a specific mitral valve evaluation aimed at extracting the more specific information required by the WHF and a 'Carpentier-style' mechanistic evaluation (see supplementary material-
Addendum **B** and Addendum C).

After the first two years of screening, the project shifted focus to become a community- based program providing HH echocardiographic screening to children at their respective schools. Only children with an abnormal screening HH study (defined as an MR jet length ≥1.5cm, aortic regurgitation [AR] jet length ≥ 0.5 cm or any WHF morphological features of RHD, congenital or acquired heart disease) underwent the same comprehensive study at TBH as previously described. Each screened case was reviewed and reported on-site by an expert, experienced in the echocardiographic evaluation of RHD (LDH, AJK, AFD, MJM, GWL, PGH).

Data management and analysis strategy

All study participant data were deidentified and entered into a Google™ Cloud Platform service (Google™ Sheets). The V-scan images from each study were downloaded to a study personal computer (PC) and accessed using GE™ Gateway software. The comprehensive echocardiographic studies were loaded onto an image viewing network (GE™ ECHOPAC). Cases of congenital heart disease were excluded from further analysis for RHD. After consensus review, comprehensive scans were categorised according to the WHF criteria as having WHF-'normal', - 'borderline-' or -'definite RHD'. A 'Carpentier-style'[21] evaluation was used to create five pre-defined mechanistic groups of MR including 1) mitral valve prolapse and prolapse spectrum, 2) rheumatic based on the presence of pseudo-prolapse, 3) congenital anterior mitral valve leaflet (AMVL) cleft and fenestration, 4) inter-scallop separation (ISS)- related MR, 5) an indeterminate category. A more detailed account of the mechanistic classification and category definitions is included as a supplement (see supplementary material -Addendum C). In cases where unanimity regarding diagnosis could not be obtained, an independent reviewer was appointed to adjudicate with the view to reaching a final diagnosis.

Statistical analysis

Data were entered into an Excel 2019 database (Microsoft), and statistical analysis was conducted in Stata 15 (StataCorp 2017). Descriptive statistical analysis was undertaken, and the prevalence of WHF 'screen-detected' RHD estimated with a 95% confidence interval (CIs). A post hoc weighted analysis was performed to more accurately assess the prevalence of RHD in the studied population. Overall survey weights were calculated to reflect the population of potential underserved children in the three district municipalities. The overall weights were based on the fraction of school children sampled from the underserved population at a school level in a district municipality and secondly the fraction of children screened in the schools that were sampled. This weighting was done at a district municipality level, and each child got the same weight based on area. The realisation rates within schools were low in all three locations, and hence the weights are large. The reference data and post hoc weights are included in the supplementary material (see supplementary material- Addendum D). Basic descriptive tables were done for describing the characteristics of the WHF- and mechanistic MR-assessment. A stratified post hoc weighted analysis was done using the survey commands of Stata 15 (StataCorp 2017). The RHD outcome was analysed using the three diagnostic categories of the screening criteria (WHF 'definite-', 'borderline-RHD' and 'normal') The breakdown of WHF 'definite-' and 'borderline-RHD' into their respective diagnostic subcategories (WHF 'definite RHD' subcategory A-D and WHF 'borderline RHD' subcategory A-C; see Table 1. 1) led to very sparse data and this level of detail could not be formally analysed. Descriptive tables of each factor (sex and location) according to RHD outcome were done, and 95% confidence intervals were calculated for the prevalence of the RHD categories. The Adjusted Wald test was used to test for associations. A survey multinomial regression model of RHD outcome on district municipality and sex was done with the

WHF 'normal' category used as a reference category. Relative Risk Ratios (RRR) were estimated and reported with 95% confidence intervals.

4.3. Results

The descriptive data and estimated prevalence of echocardiographic RHD in each screened district municipality are presented in Tables

Table 4. 1. A total of 5225 secondary school children (aged 13-19) were enrolled in the study. Of these, 3474 children (66.4%) from the City of Cape Town-, 923 children (17.6%) from the Drakenstein- and 828(15.8%) children from the Stellenbosch-municipalities were screened. The mean age of screened schoolchildren was 15 years (standard deviation [SD], 2 years). There was a female predominance in participants from all district municipalities, ranging from 61.9%-66.6% of children screened. None of the enrolled study participants gave a history of a previous diagnosis of acute rheumatic fever (ARF) or RHD. A total of 49 WHF 'definite RHD' and 104 'borderline RHD' cases were detected by echocardiography. Overall, the estimated prevalence was 9.1 cases /1000 population (95% CI, 6.8 to 12.1) for WHF 'definite RHD' and 19.5 cases per 1000 population (95% CI, 16.0 to-23.7) for WHF 'borderline RHD'. Overall, 97.1% of children screened normal for RHD. The pattern of disease involvement for WHF 'definite RHD' and WHF 'borderline RHD' cases is presented in Table 4. 2. Children identified with WHF 'definite RHD' Subcategory A (isolated rheumatic MV disease) constituted the majority (n=39; 79.6%) of WHF 'definite RHD' cases identified in our study cohort. Screened cases identified with WHF 'borderline RHD' Subcategory B (isolated 'pathological' MR) contributed the majority (n=68; 65.4%) of WHF 'borderline RHD' cases. The echocardiographic findings in children identified with WHF 'definite RHD' are presented in Table 4. 3. The MV criteria that contributed to the majority of WHF 'definite RHD' diagnoses were anterior mitral valve leaflet (AMVL) thickening (57.1%), MV restriction (83.7%), excessive leaflet tip motion (85.7%) and WHF 'pathological' MR (85.7%). Chordal thickening was identified in a minority of cases (4%). The aortic valve (AV) criteria that contributed to the majority of WHF 'definite RHD' were irregular/focal thickening (18.4%), restricted leaflet motion (20.4%) and WHF 'pathological' AR (14.3%). An AV coaptation defect was identified in a single case (2%) with no cases (0%) of AV prolapse identified. Exclusion of the criteria that were least utilised in a WHF 'definite RHD' diagnosis (i.e. chordal thickening, coaptation defect and AV prolapse), did not result in a reclassification of a borderline or definite case.

The results of a mechanistic evaluation of MR in WHF 'borderline RHD' cases with isolated WHF 'pathological' MR are presented in Table 4. 4. A mechanistic evaluation of MR in these cases allowed for further classification in 54 children (79.4%). Here, ISS-related MR was identified as the underlying mechanism of MR in 48 children (70.5%). In 29 children (60.4%), MR was seen to originate from an ISS in the P2/P3 region of the PMVL (data not shown). The mechanism of MR could not be classified into one of the pre-determined categories (indeterminate) in 14 cases (20.6%). The variables

associated with ‘screen-positive’ RHD are depicted in Table 4. 4. A multinomial regression model identified female sex as the only independent predictor of WHF ‘definite RHD’ (RRR= 2.4; 95% CI, 1.19-4.99; p= 0.015).

Sixty-seven children (1.25%) were identified with congenital heart anomalies. The majority of which were minor with 13 patients requiring further therapy or long-term follow up. This included four cases of atrial septal defect requiring closure, four cases of hypertrophic cardiomyopathy, two cases of patent ductus arteriosus requiring closure, two cases of bicuspid aortic valve disease and a single case of a cleft AMVL. All ‘screen-positive’ children were entered into an EIA RHD register for long-term surveillance. All children with WHF ‘definite RHD’ were counselled (together with a parent/caregiver) and offered secondary prophylaxis.

4.4. Discussion

This is the largest echocardiographic RHD screening study reported in South Africa. Our findings contribute much-needed data that highlight a heavy burden of latent RHD amongst high-risk school children in this region. In addition to ongoing EIA screening efforts, we sought to evaluate novel screening techniques that could improve the specificity of the current WHF guideline. The impact of a novel assessment, tailored to determine the underlying mechanism of MR was significant. In our study, ISS-related MR was identified in almost half of the children classified with WHF ‘borderline RHD’ and over 70% of cases with isolated ‘pathological’ MR. The findings of this study add credence to our hypothesis that a mechanistic MR assessment could significantly reduce the number of cases ‘misclassified’ with WHF ‘borderline RHD’.

EIA is the second RHD screening study to be conducted in the Western Cape province of South Africa. In 2015, Engel et al. compared the prevalence of echocardiographic RHD amongst 2720 school children in Bonteheuwel and Langa; two adjacent residential areas within the City of Cape Town municipality.[5] The study reported a WHF ‘definite RHD’ prevalence of 4.8/1000 and an overall subclinical disease prevalence of 20.2/1000, establishing subclinical RHD as an endemic condition amongst select high-risk populations in the Western Cape. Our findings of a WHF ‘definite RHD’ prevalence of 9.1/1000 and an overall WHF subclinical disease prevalence of 28.6/ 1000 supports data that RHD remains a significant health challenge amongst high-risk children living in this region.

While the reported prevalence of subclinical RHD in our cohort was higher than that studied in Bonteheuwel and Langa, the variation was not statistically significant. There were, however, notable differences in the echocardiographic pattern of WHF ‘definite RHD’ identified in both studies. In the previous study in Cape Town, concomitant borderline lesions affecting both the MV and AV comprised the majority (76.9%) of reported WHF ‘definite RHD’.[5] In contrast, the children in our cohort demonstrated more ‘severe’ lesions, predominantly affecting the MV (79.6%) and to a far lesser

extent, the AV (14.3%) and borderline lesions of the MV and AV (6.1%; Table 4. 2) The reason for the differences in the reported prevalence and pattern of valve disease between the studies is not apparent. Various factors may have contributed to these findings that include differences in the acquisition protocol between the two studies as well as the application and interpretation of the WHF criteria. This speaks to the complexity of applying the current criteria consistently despite having a guideline aimed at standardising assessment and interpretation.

The sex-ratio in our studied cohort was predominantly female (65.8%). A similar proportion was described in the previous echocardiographic study in Cape Town, where 58.9% of enrolled participants were female. This finding could, in part, be explained by a documented trend of high drop-out rate amongst males attending South African secondary schools in low socioeconomic communities.[22] From the investigators perspective, it appeared that the diligence with which consent forms were returned was higher amongst females than amongst males. In a multivariate model, female sex was identified as the only independent predictor of WHF 'definite RHD' with a relative risk of 2.4 (Table 4. 5). These findings mirror those of a recent RHD screening study in South East Asia (Timor Leste).[23] While the underlying cause for this sex-based difference is unknown, it is consistent with the known association between female sex and mitral stenosis (MS); the single most specific valve lesion for RHD.[24,25]

The development of the WHF guideline represents an initial step to deciphering the natural history and appropriate management of subclinical RHD. It is imperative that a process of critical appraisal and potential revision of the criteria is established to ensure that further progress is made.

Based on our EIA experience, we have identified aspects within the current WHF morphological assessment that require scrutiny. Firstly, we have found that the reproducibility of an AMVL thickness assessment based on a leaflet measurement with a strict cut-off is poor and have sought to investigate whether other non-measurement-based methodologies can effectively identify typical rheumatic-related leaflet thickening.[26] Secondly, we have introduced strict, independent definitions of MV and AV restriction to improve detection and reproducibility of this essential morphological feature of RHD. Lastly, in our experience, we find that the current WHF MV criterion of 'excessive leaflet tip motion' is ambiguous and requires further clarification. Currently, the WHF definition describes excessive leaflet tip motion as "*displacement of the tip or edge of an involved leaflet towards the left atrium resulting in abnormal coaptation and regurgitation*".[1] We recognise that this definition encompasses the well-documented finding of excessive leaflet motion in patients with ARF where the underlying mechanism is related to primary chordal rupture.[1] However, this particular mechanism was not encountered in any of the 5225 children screened in our program. It is our experience that this current definition, juxtaposed with the WHF's requirement to exclude cases with mitral valve prolapse (defined as atrial displacement of any portion of the mitral valve ≥ 2mm below the MV annulus in a long-axis view)[27] obscures the identification of a frequently encountered RHD-related leaflet abnormality in our screening program. Here, we recognise that 'excessive leaflet tip motion' includes

the description of ‘pseudoprolapse’ of the AMVL. Here, the tip of the AMVL appears to move excessively relative to the PMVL tip, but importantly, remains above the annulus. The underlying mechanism is typically not true excessive AMVL tip prolapse but rather represents a degree of PMVL restriction. This form of ‘excessive leaflet tip motion’ was indeed frequently encountered in the EIA screening population (and reported here as such) in patients with other morphological features of RHD, supporting its well-known association with RHD in patients with clinically significant rheumatic MR.[28] In comparison, the infrequent finding of true AMVL prolapse and prolapse spectrum was not associated with other morphological features of RHD in this study (Table 4. 4).

In this study, we evaluated the frequency with which each specific WHF criterion contributed to a WHF ‘definite RHD’ diagnosis. This process was performed to gain further insight into the diagnostic weight that each echocardiographic feature may carry (Table 4. 3). ‘Pathological’ valve regurgitation was, not unexpectedly, a prominent finding in both AV and MV disease, since a ‘pathological’ functional deficit is a prerequisite of the diagnosis in WHF ‘definite RHD’. While thickening, restricted leaflet motion and excessive leaflet tip motion (‘pseudoprolapse’ of the AMVL) were the predominant features contributing to a definite diagnosis, we noted that there were morphological criteria that were less frequently identified in our evaluation. These include the identification of chordal thickening in MV assessment (two cases) and the presence of a coaptation defect (one case) as well as prolapse in the AV assessment (0 cases). Interestingly, the complete removal of these criteria did not result in a reclassification of any cases previously included in the WHF ‘definite RHD’ group, suggesting that at least in our cohort, these criteria may be redundant. Further study and corroboration from other screening databases are required to test the validity of this finding.

WHF ‘borderline RHD’ constituted the majority (67.9%) of ‘screen-detected’ RHD identified in this study (Table 4. 2). This specific WHF RHD ‘demographic’ is consistent with the findings published by the majority of large-scale RHD screening studies where borderline disease constitute between 54%-88% of echocardiographic RHD.[2-5,16] There is a concern that the borderline group represents a diverse spectrum that includes RHD, but owing to a reduction in diagnostic specificity may equally well contain cases of alternate ‘pathologies’ or even normal variants considered on the ‘upper limit of normal’.[32,33] Several studies have sought to report on the outcome of this group; however, the utility of long-term data on the borderline group remains contentious, mainly if so much uncertainty exists about what actually constitutes the group and whether RHD even comprises the majority. Furthermore, a large borderline group is a fundamental impediment to the success of large-scale screening studies as it significantly increases the total number of cases requiring re-reading for further scrutiny. It is therefore critical that effort is directed at reducing the size of this diagnostic category.

Two rational approaches could be taken to improve the specificity of the current screening criteria, particularly in the assessment of borderline cases. The first approach is to downgrade the importance of an MR severity assessment in the screening criteria in favour of criteria with an emphasis on a morphological RHD assessment. The second approach to potentially improving the accuracy of the

current screening criteria would be to address the diagnostic criterion contributing to the bulk of borderline disease identified in RHD screening programs, namely, isolated 'pathological' MR. Of the 104 cases identified in our study with WHF 'borderline RHD', 68 cases (65.4%) were diagnosed with isolated 'pathological' MR (WHF 'borderline RHD'- subcategory B). Similarly, this subcategory constitutes a significant proportion (33-88%) of reported borderline disease in published studies.[2-5,16] We have previously identified that the WHF's incorporation of a Doppler-based functional assessment hampers the identification of true rheumatic disease.[33,34] The Doppler criteria, while providing a standardised method of MR classification, remains a non-specific tool providing no additional information with regards to the underlying aetiology of the valvular dysfunction.

A novel strategy implemented in EIA was to include a mechanistic assessment of MR to determine whether other, non-RHD patient groups might be misclassified with borderline disease. (see supplementary material- Addendum C) This led to the identification of the ISS, an anatomical variant of the PMVL and a ubiquitous finding amongst our screened low- and high-risk cohorts. The frequency with which we identified ISS-related MR as the underlying mechanism of incompetence in WHF 'borderline RHD' raised the question as to whether a possible non-rheumatic entity may be causative. We have recently reported the prevalence of ISS-related MR in a cohort of high-and very low-risk South African school children. In this preliminary investigation, ISS-related MR was identified in a similar proportion of children; contributing between 78.5% -83.3% of cases with isolated 'pathological' MR (see Chapter 3; Inter-scallop separations of the posterior mitral valve leaflet: a solution to the 'borderline RHD' conundrum?). In the current study, we report ISS-related MR as the predominant mechanism in 70.5% of cases with isolated WHF 'pathological' MR representing almost half of those identified with WHF 'borderline RHD'(Table 4. 3). The findings of the current study mirror those reported in our preliminary investigation and underscore the value of a mechanistic evaluation to investigate screened cases with MR further. Ongoing research is required to determine whether our findings are unique to our screened demographic and if they can be reproduced by screening programs working elsewhere in Africa. It is critical that a longitudinal study of participants identified with ISS-related MR be prioritised to determine the natural evolution of this entity. The outcome of these studies could improve our understanding of the natural history of true subclinical RHD.

Limitations

The primary aim of EIA is to provide RHD screening for schoolchildren living in underserved communities of the Western Cape. Consequently, schools sampled in each area did not constitute a random sample but represented schools that had given access to the initiative. Although we aimed to screen all children in each respective school, the percentage of children with informed consent was relatively low. These two sampling components may lead to bias in our study results.

After the first two years of EIA, the validation study was omitted in screen-negative cases. In the absence of a validating comprehensive study, it is conceivable that some cases of RHD were missed

and erroneously misclassified as 'normal'. However, the data from the first two years of comparing HH to comprehensive scans in EIA (data not shown) demonstrated good concordance validating the decision only to perform detailed scans in 'screen-positive' or 'screen-uncertain' individuals. This has subsequently been validated in other published series[35] and has become standard in most large-scale RHD screening programs.

4.5. Conclusion

Latent RHD remains a significant health challenge in underserved communities within the Western Cape, South Africa. However, the role of RHD screening as a viable means of secondary prevention of RHD in endemic regions remains controversial. The current WHF criteria identify a large, heterogenous borderline cohort with potential disease that has complicated the interpretation of outcome studies and hampered the implementation of population-based RHD screening. In this study, the incorporation of a mechanistic evaluation of MR emerged as a potential solution to reducing the size of the borderline group. Here, an ISS – a common, normal feature of the PMVL was identified as the underlying mechanism of isolated WHF 'pathological' MR in a significant proportion of borderline cases. Further study will determine the natural history of this entity, which appears to be a ubiquitous finding in otherwise normal hearts and inform the role of a novel screening strategy for improving the specificity of the current guideline.

Acknowledgements

The Echo in Africa project is funded by an Edwards Lifesciences 'Every heartbeat matters' grant (#10137553). We thank the Western Cape Department of Education, principals, governing bodies and communities of each respective school for their ongoing support. The EIA investigators would like to thank General Electric for providing echocardiographic equipment for this program. We acknowledge the BSE and their members who volunteer their time and echocardiographic skills for this project, without whom this research could not have been possible.

4.6. References

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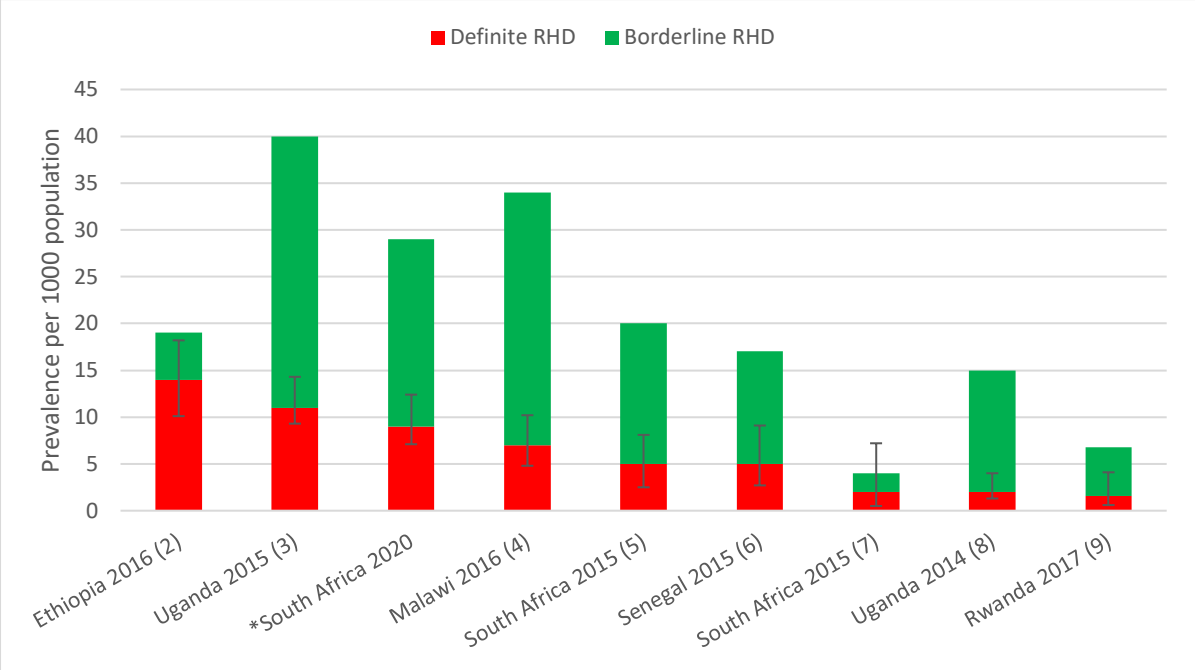
4.7. Figures

Figure 4. 1. Prevalence of rheumatic heart disease in screened African populations by World Heart Federation criteria



*Current study

RHD, rheumatic heart disease

4.8. Tables

Table 4. 1. Summary statistics from the Echo in Africa project (2014-2018)

	Echo in Africa (Total) (n=5225)	City of Cape Town (n=3474)	Drakenstein (n=923)	Stellenbosch (n=828)
Characteristics				
Mean age (SD)	15.0 (2.0)	15.0 (2.0)	15.5 (2.0)	15.0 (2.0)
Female gender (n, %)	3439 (65.8)	2316 (66.6)	572 (61.9)	551 (66.5)
Prevalence of WHF RHD (weighted no. of cases/1000 [95% CI])				
WHF 'definite RHD'	9.1(6.8-12.1)	8.3(5.8-12.0)	13 (7.4-22.8)	9.7(4.8-19.2)
WHF 'borderline RHD'	19.5(16.0-23.7)	19.9(15.7-25.2)	15.2(9.0-25.5)	21.7(13.7-34.3)
Total WHF RHD	28.6(24.3-33.5)	28.2(23.2-34.3)	28.2(19.2-41.1)	31.4(21.5-45.7)

SD, standard deviation; WHF, World Heart Federation; RHD, rheumatic heart disease; CI, confidence inter

Table 4. 2. Pattern of WHF echocardiographic valve disease

	Echo in Africa (Total)	City of Cape Town	Drakenstein	Stellenbosch
Definite cases	**N=49**	**N=28**	**N=12**	**N=9**
(A) 'Pathological' MR and at least two morphological features of RHD of the MV, n (%)	39 (79.6)	25 (89.3)	6 (50)	8 (88.9)
(B) MS with mean gradient >4 mm Hg	0	0	0	0
(C) 'Pathological' AR and at least two morphological features of RHD of the AV, n (%)	7 (14.3)	2 (7.1)	4 (33.3)	1 (11.1)
(D) Borderline disease of both the AV and MV, n (%)	3 (6.1)	1 (3.6)	2 (16.7)	0 (0)
Borderline cases	**N=104**	**N=65**	**N=20**	**N=19**
(A) At least two morphological features of RHD of the MV without 'pathological' MR or MS, n (%)	20 (19.2)	17 (26.2)	2 (10)	1 (5.3)
(B) 'Pathological' MR, n (%)	68 (65.4)	39 (60)	16 (80)	13 (68.4)
(C) 'Pathological' AR, n (%)	16 (15.4)	9 (13.8)	2 (10)	5 (26.3)

WHF, World Heart Federation; MR, mitral regurgitation; MV, mitral valve; MS, mitral stenosis; AR, aortic regurgitation; AV, aortic valve; RHD, rheumatic heart disease

Table 4. 3. Echocardiographic findings in children with WHF 'definite RHD'

Echocardiographic finding		
	n	**%**
Morphological MV		
AMVL thickening ≥3mm	28	57.1
Chordal thickening	2	4
Restricted leaflet motion	41	83.7
Excessive leaflet tip motion	42	85.7
MR		
WHF 'pathological' MR	42	85.7
Morphological AV		
Irregular/focal thickening	9	18.4
Coaptation defect	1	2
Restricted leaflet motion	10	20.4
Prolapse	0	0
AR		
WHF 'pathological AR'	7	14.3

WHF, World Heart Federation; RHD, rheumatic heart disease; MV, mitral valve; AMVL, anterior mitral valve leaflet; MR, mitral regurgitation; AV, aortic valve; aortic regurgitation, AR

Table 4. 4. Mechanism of MR in WHF 'borderline RHD' cases with isolated 'pathological' MR

	Echo in Africa (Total)	City of Cape Town	Drakenstein	Stellenbosch
Mechanism of MR	N= 68	N=39	N=16	N=13
ISS n, %	48 (70.5)	25 (64.1)	13 (81.3)	10 (76.9)
AMVL cleft n, %	1 (1.5)	0 (0)	1 (6.3)	0 (0)
MVP/MVPS n, %	5 (7.4)	4 (10.3)	1 (6.3)	0 (0)
Pseudo-prolapse of AMVL	0	0	0	0
Indeterminate n, %	14 (20.6)	10 (25.6)	1 (6.3)	3 (23.1)

ISS, inter-scallop separation; AMVL, anterior mitral valve leaflet; MVP/MVPS, mitral valve prolapse and mitral valve prolapse spectrum,

Table 4. 5. Univariate and multivariate analysis of RHD diagnosis by sex and district municipality

		WHF 'definite RHD'					WHF 'borderline RHD'			
Factor	p-value for RHD	Univariate RRR (95% CI)	*P-value*	Multivariate RRR (95% CI)	*P-value*		Univariate RRR (95% CI)	*P-value*	Multivariate RRR (95% CI)	*P-value*
Sex	0,026									
Male		1		1			1		1	
Female		2.37(1.17-4.82)	0,018	2.43 (1.19-4.99)	0,015		1.05(0.68-1.61)	0,823	1.06 (0.69-1.64)	0,771
Location	0,617									
City of Cape Town		1		1			1		1	
Drakenstein		1.56(0.79-3.07	0,200	1.79 (0.88-3.67)	0,100		0.76(0.43-1.36)	0,362	0.86 (0.48-1.56)	0,636
Stellenbosch		1.16(0.53-2.55)	0,710	1.18(0.53-2.63)	0,670		1.10(0.65-1.86)	0,727	1.12 (0.66-1.89)	0,669

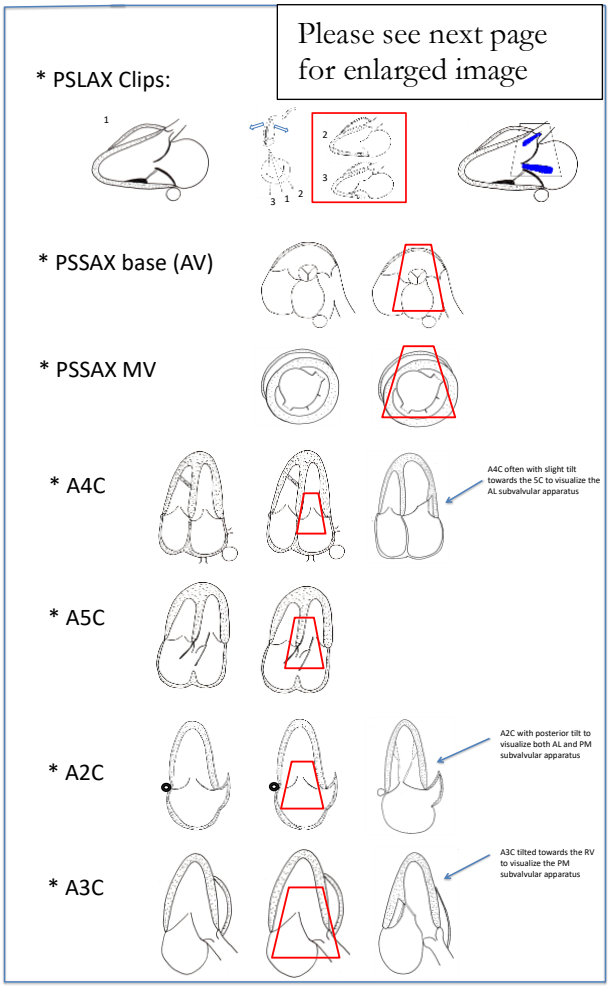
WHF, World Heart Federation; RHD, rheumatic heart disease; RRR, relative risk ratio; CI, confidence interval

4.9. Supplementary material

Addendum A

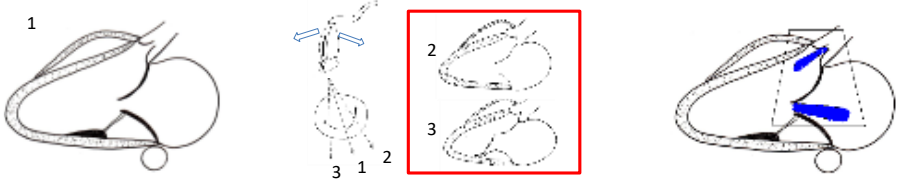
Diagrammatic representation of the handheld (HH) screening protocol

Seven cardiac views (A-G) are incorporated into the screening transthoracic echocardiogram (TTE). The use of colour Doppler is indicated by the red-colour box in each cardiac view. In the parasternal long-axis (PSLAX) view (A), a 'parasternal sweep' is performed from the neutral position in (1), tilting the probe upwards and downwards to evaluate the lateral (2) and medial portion (3) of the valve respectively. This step should be performed with and without colour Doppler.

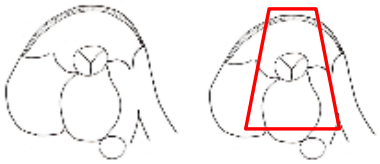


PSLAX, parasternal long-axis; PSSAX, parasternal short-axis; AV, aortic valve; MV, mitral valve; A4C, apical four-chamber; A5C, apical five-chamber view; A2C, apical two-chamber; A3C, apical three-chamber; AL, anterolateral; PM, posteriomedial

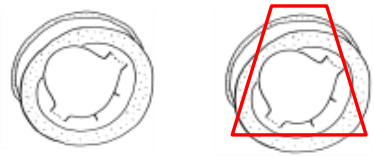
* PSLAX Clips:



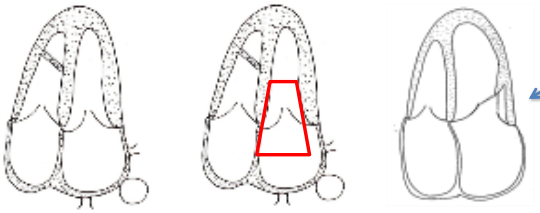
* PSSAX base (AV)



* PSSAX MV

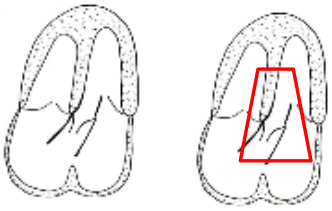


* A4C

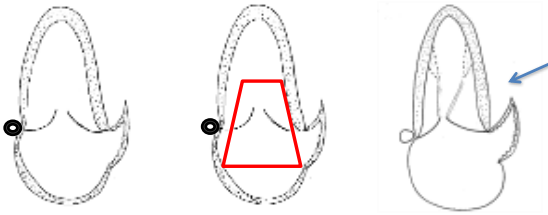


A4C often with slight tilt towards the 5C to visualize the AL subvalvular apparatus

* A5C

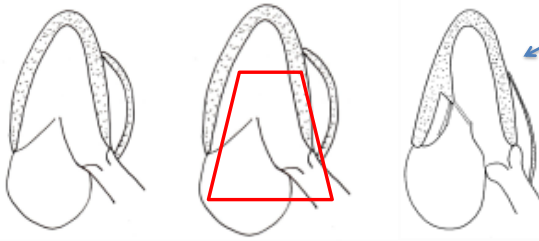


* A2C



A2C with posterior tilt to visualize both AL and PM subvalvular apparatus

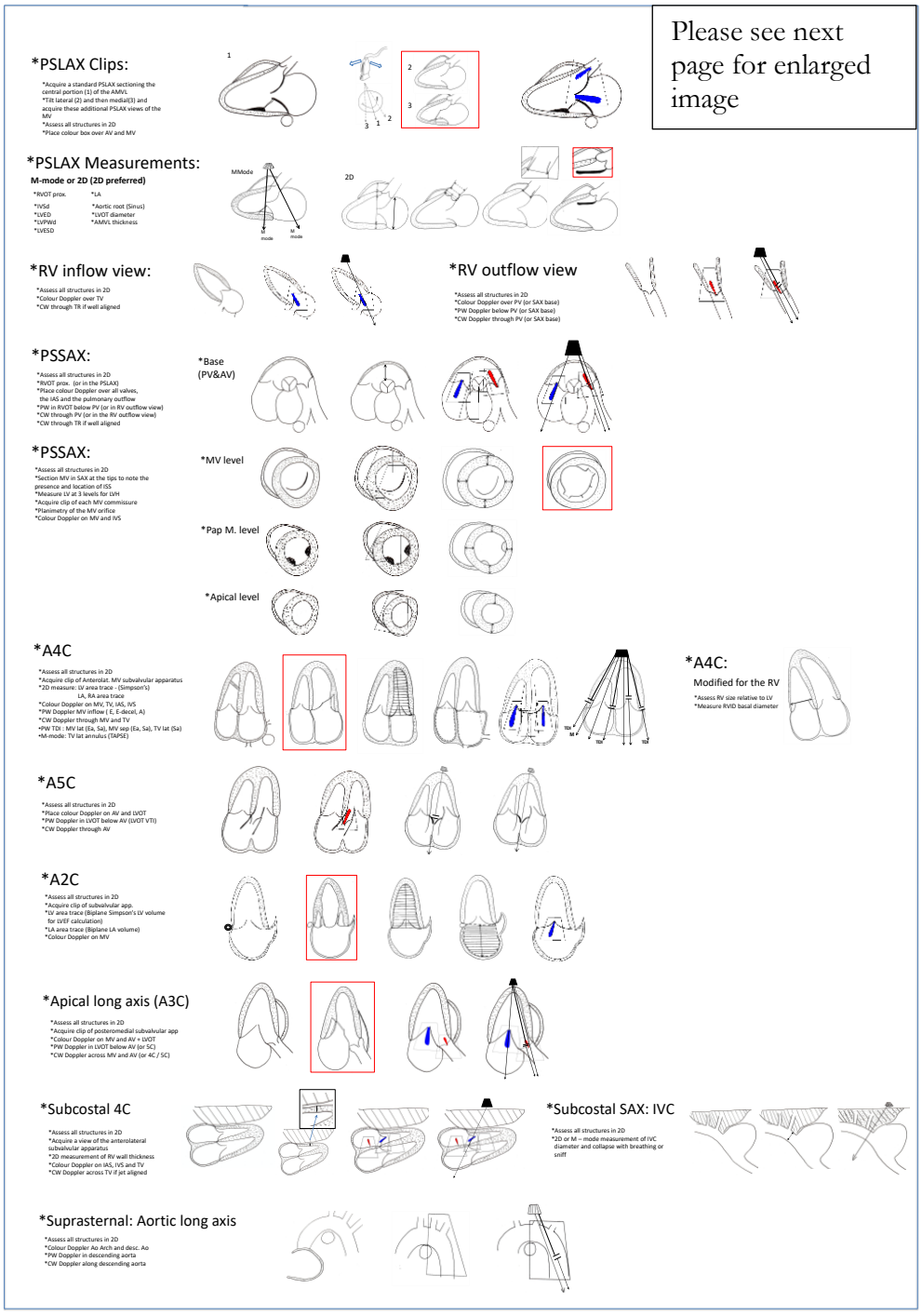
* A3C



A3C tilted towards the RV to visualize the PM subvalvular apparatus

Addendum B

Diagrammatic representation of the transthoracic (TTE) study protocol



*PSLAX Clips:

*Acquire a standard PSLAX sectioning the central portion (1) of the AMVL
*Tilt lateral (2) and then medial(3) and acquire these additional PSLAX views of the MV
*Assess all structures in 2D
*Place colour box over AV and MV

*PSLAX Measurements:

M-mode or 2D (2D preferred)

*RVOT prox.
*IVSd
*LVED
*LVPWd
*LVESD
*LA
*Aortic root (Sinus)
*LVOT diameter
*AMVL thickness

*RV inflow view:

*Assess all structures in 2D
*Colour Doppler over TV
*CW through TR if well aligned

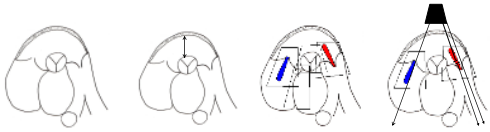
*RV outflow view

*Assess all structures in 2D
*Colour Doppler over PV (or SAX base)
*PW Doppler below PV (or SAX base)
*CW Doppler through PV (or SAX base)

*PSSAX:

*Assess all structures in 2D
*RVOT prox. (or in the PSLAX)
*Place colour Doppler over all valves, the IAS and the pulmonary outflow
*PW in RVOT below PV (or in RV outflow view)
*CW through PV (or in the RV outflow view)
*CW through TR if well aligned

*Base (PV&AV)



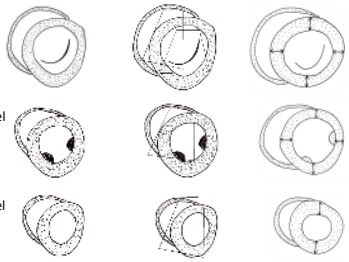
*PSSAX:

*Assess all structures in 2D
*Section MV in SAX at the tips to note the presence and location of ISS
*Measure LV at 3 levels for LVH
*Acquire clip of each MV commissure
*Planimetry of the MV orifice
*Colour Doppler on MV and IVS

*MV level

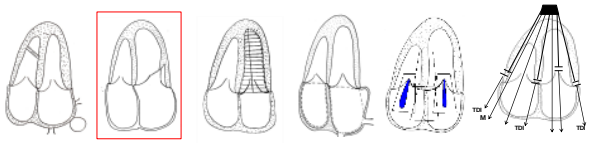
*Pap M. level

*Apical level



*A4C

*Assess all structures in 2D
*Acquire clip of Anterolat. MV subvalvular apparatus
*2D measure: LV area trace - (Simpson's) LA, RA area trace
*Colour Doppler on MV, TV, IAS, IVS
*PW Doppler MV inflow (E, E-decel, A)
*CW Doppler through MV and TV
•PW TDI : MV lat (Ea, Sa), MV sep (Ea, Sa), TV lat (Sa)
•M-mode: TV lat annulus (TAPSE)



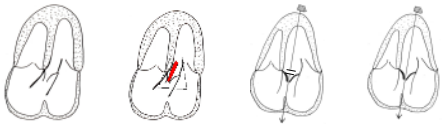
*A4C:

Modified for the RV

*Assess RV size relative to LV
*Measure RVID basal diameter

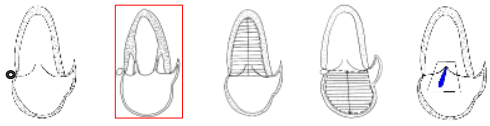
*A5C

*Assess all structures in 2D
*Place colour Doppler on AV and LVOT
*PW Doppler in LVOT below AV (LVOT VTI)
*CW Doppler through AV



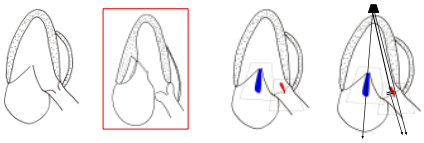
*A2C

*Assess all structures in 2D
*Acquire clip of subvalvular app.
*LV area trace (Biplane Simpson's LV volume for LVEF calculation)
*LA area trace (Biplane LA volume)
*Colour Doppler on MV



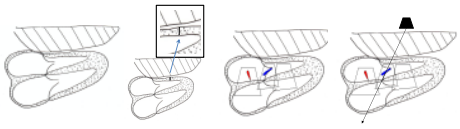
*Apical long axis (A3C)

*Assess all structures in 2D
*Acquire clip of posteromedial subvalvular app
*Colour Doppler on MV and AV + LVOT
*PW Doppler in LVOT below AV (or 5C)
*CW Doppler across MV and AV (or 4C / 5C)



*Subcostal 4C

*Assess all structures in 2D
*Acquire a view of the anterolateral subvalvular apparatus
*2D measurement of RV wall thickness
*Colour Doppler on IAS, IVS and TV
*CW Doppler across TV if jet aligned



*Subcostal SAX: IVC

*Assess all structures in 2D
*2D or M – mode measurement of IVC diameter and collapse with breathing or sniff

*Suprasternal: Aortic long axis

*Assess all structures in 2D
*Colour Doppler Ao Arch and desc. Ao
*PW Doppler in descending aorta
*CW Doppler along descending aorta

Addendum C

Mechanistic evaluation of MR in RHD screening

A 'Carpentier-style'[21] classification of mitral valve regurgitation was used to identify the following mechanisms relevant to our screening population:

1. **Normal leaflet motion**

Leaflets with normal motion were categorised into those with a mitral regurgitation (MR) mechanism attributable to an underlying inter-scallop separation (ISS; MR originating from slit-like separations between the scallops of the posterior mitral valve leaflet [PMVL]) or a cleft involving the anterior mitral valve leaflet (AMVL). The origin of an ISS-related MR jet is confirmed on an optimised parasternal short-axis (PSSAX) view (ensuring to section the tips of the mitral valve leaflet). Typically, the MR jet is appreciated at, or immediately adjacent to the ISS as a fixed spot of colour or seen to be moving in a vertical up-down fashion through the PMVL rather than across the line of valvular coaptation during systole.

2. **Excessive leaflet motion**

For this evaluation, leaflets with excessive motion were further categorised into cases with either mitral valve prolapse (MVP) or MVP-spectrum. MVP was diagnosed when the leaflet was seen to move beyond the annular plane (>2mm) in a long-axis orientation, in keeping with current consensus guidelines.[92] MVP-spectrum was diagnosed in cases where some portion of the leaflet was seen to move beyond the annular plane with associated tip malcoaptation. In these cases, there was no associated PMVL restriction, nor was the valve seen to prolapse >2mm beyond the annular plane in a long-axis orientation. Typically, the MR jet is seen to emanate across the line of valvular coaptation, exhibiting a broad colour Doppler jet on the optimised PSSAX view.

3. **Restricted leaflet motion**

3.1 Systolic and diastolic restriction of the PMVL with resultant malcoaptation of the PMVL and AMVL during systole gives the impression of AMVL 'tip prolapse' or 'excessive leaflet motion'. These terms are synonymous and generate so-called AMVL 'pseudoprolapse' which cannot be regarded as true prolapse, as the AMVL is seen to be in its normal position at end-systole and does not cross the annular plane.[27] Pseudoprolapse of the AMVL generates the characteristic posteriorly directed jet of rheumatic MR with a similar broad Doppler jet exhibited on the optimised PSSAX view.

3.2 Restricted PMVL motion primarily during systole ('tethering') has a wide differential and includes any aetiology known to alter the geometry of the left ventricle. This category is not likely to be encountered during screening amongst asymptomatic children.

4. **Indeterminate**

Screened cases with MR whose underlying mechanism was not clearly discernible were classified as 'indeterminate'.

Addendum D

Reference data* and post hoc weights in the three study areas

Municipality	Total number of secondary school children	Number of underserved children	Number of children in schools sampled	Number of children enrolled	Sampling fraction of schools	Sampling realisation within schools	weight
City of Cape Town	235400	103000	15000	3474	0.146	0.232	29.65
Drakenstein	26700	18000	6000	923	0.333	0.154	19.50
Stellenbosch	27600	15000	4000	828	0.267	0.207	18.12
Total	289700	136000	25000	5225	0.184	0.209	

*Reference data obtained from annual reports published by the Western Cape Education Department (WCED)

Chapter 5: Screening for rheumatic heart disease: The reliability of anterior mitral valve leaflet thickness measurement

Chapter five is a published manuscript that sought to determine the reliability of an anterior mitral valve leaflet (AMVL) measurement assessment as prescribed by the current World Heart Federation (WHF) criteria for echocardiographic diagnosis of RHD. My role in this study included developing the study protocol with guidance from Dr CJ Lombard, the preparation of the study material and data capture with the support of B Franckeiss. The statistical analyses was performed with guidance from Dr CJ Lombard (Stellenbosch University, Division of Epidemiology and Biostatistics). I am the primary author of the manuscript included in this chapter. MJ Monaghan, GW Lloyd, AJK Pecoraro reviewed the final manuscript. AF Doubell and PG Herbst were the co-supervisor and supervisor respectively. They supervised the study design and execution. Both reviewed the final draft of the manuscript.

Published manuscript

Screening for rheumatic heart disease: The reliability of anterior mitral valve leaflet thickness measurement
LD Hunter, CJ Lombard, MJ Monaghan, GW Lloyd, AF Doubell, B Franckeiss, AJK Pecoraro, PG Herbst
Echocardiography. 2020 May; (37):808-848. doi:10.1111/echo.14751

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5.1. Abstract

Background

Studies determining the reliability of the World Heart Federation (WHF) anterior mitral valve leaflet (AMVL) measurement are limited by the introduction of bias in their test-retest analyses. This study sought to determine the reliability of the current AMVL measurement whilst controlling for systematic bias.

Methods

Retrospective analysis of echocardiographic data from 16 patients with previous acute rheumatic fever were performed. Included in this study was an optimised cine-loop of the mitral valve(MV)[reader-optimised measurement (ROM]) in the parasternal-long-axis view and an optimised still-image of the MV obtained from the same cine-loop [specialist-optimised image(SOI)] Each still image and associated cine-loop was quadruplicated and randomised to determine intra-and inter-rater agreement and quantify the impact of zoom on AMVL measurement.

Results

SOI without zoom reflected the highest degree of agreement in both cohorts with an ICC of 0.29 and 0.46. The agreement in ROM images without zoom was ICC of 0.23 and 0.45. The addition of zoom to SOI decreased agreement further to an ICC 0.20 and 0.36. The setting associated with the poorest agreement profile was ROI with zoom with an ICC of 0.13
and 0.34 respectively. The intra-rater agreement between readers in both cohorts was moderate across all settings with an ICC ranging between 0.64- 0.86.

Conclusions

The WHF AMVL measurement is only moderately repeatable within readers and demonstrates poor reproducibility that was not improved by the addition of a zoom-optimised protocol. Given our study findings, we cannot advocate the current WHF AMVL measurement as a reliable assessment for RHD.

5.2. Introduction

Abnormal thickening of the mitral valve (MV) and associated chordal tissue is a prominent feature of rheumatic heart disease (RHD).[1] The World Heart Federation (WHF) screening criteria for the echocardiographic diagnosis of RHD advocate the assessment and measurement of the anterior mitral valve leaflet (AMVL).[1] The WHF guideline stipulates that the thickest portion of the AMVL should be measured during diastole (with the leaflet at full excursion) in a frame with maximal separation of chordae from the leaflet tissue. The measurement should be performed on an image that was acquired without harmonic imaging with the gain settings optimised for adequate image resolution.[1] Post-mortem studies in children (aged<20 years) with normal hearts have established the reference range of the AMVL to be between 0.5-2.3mm with significant differences noted between the mean measurement at various predetermined sites of the AVML.[2] Consequently, the current AMVL assessment requires the reader to consistently perform one of the smallest measurements in echocardiography on a structure that demonstrates an inherent, asymmetrical variability in thickness.[3,4]

Recent validation studies have demonstrated the WHF AMVL measurement to be a reliable assessment with a high degree of repeatability[5](intra-rater agreement) and reproducibility[5](inter-rater agreement) with an interclass and intraclass correlation coefficient (ICC) ranging between 0.75-0.85 and 0.79-0.90 respectively.[6,7] However, there is a disparity between the reproducibility demonstrated in these studies and those published in preceding screening studies.[8,9] An important consideration remains whether these studies accurately capture the complexity of ‘real-world’ AMVL measurement

or whether simplifying the measurement technique in study methodology has introduced an important bias.

The variation introduced by various technical and pathological factors has been outlined in a recent critique of the WHF criteria.[3] Whilst the role of some variables are well described, i.e. the relationship between harmonic-enhanced images and measured thickness, there are some factors that are poorly understood and are not addressed by the current guideline including the use and role of a zoom-optimised measurement.[1,3]

The aim of this study was to perform a reliability analysis of the WHF AMVL measurement according to best practise as outlined in current literature.

5.3. Methods

Study design and participants

This was a retrospective study using a random selection of echocardiography studies obtained from an acute rheumatic fever (ARF) database, managed at the Tygerberg Academic Hospital, Western Cape, South Africa. This registry comprises of enrolled subjects from three national provinces (Western Cape, Eastern Cape and Kwa-Zulu Natal) with a documented history of ARF according to the Jones or Modified Jones criteria.[10] Ethics approval was obtained from the relevant Health Sciences committee at the University of Stellenbosch (S17/02/030).

Echocardiographic evaluation

All routine echocardiographic studies were captured between November 2017 and December 2018 by an experienced echocardiographer, specialised in RHD screening and identification (LDH). A GE Vivid I laptop machine (General Electric Vivid I, Milwaukee, WI, USA) with a 2- to 3.6 MHz transducer probe (GE 3S) was used to obtain each comprehensive assessment. All studies were performed and reported in accordance with the requirements stipulated in the WHF guideline.[1]

Image selection and randomisation process

The process of case selection and subsequent randomisation of cine-loops/still-images are summarised in Figure 5. 1. A detailed explanation is as follows: all patients from the registry who had undergone a previous MV procedure (surgical repair or replacement) were excluded from study inclusion. All remaining studies were anonymised and allocated a unique study number (i.e. 1-100, etc). A simple random sample without replacement of 16 studies were selected with the use of a random number generator.

The following clips/images were obtained by the lead investigator (LDH) from each selected study:

1. Two-beat cine-loop without harmonics in the parasternal long-axis view (PSLAX) sectioning the central portion of the MV leaflet (A2 segment). The subsequent measurements obtained from this cine-loop represent reader-optimised images and an 'in-the-field' measurement.

2. Optimised PSLAX still-image from the identical cine-loop as described in (1). The subsequent measurements obtained from this still-image represent a specialist-optimised image. Moreover, the reliability profile obtained from this evaluation will represent the 'gold standard' and will create a reference point with which to compare the contribution of the reader in (1). The specific pre-selected still-image was captured according to the methodology incorporated by Webb et al.[6] (i.e. when the AMVL was approximately parallel to the ventricular septum with maximal separation from overlying chordal tissue.)

We sought to determine the impact of zoom on the reliability profile in each measurement protocol. Therefore each cine-loop and still-image clip was quadruplicated (to quantify the impact of zoom on AMVL measurement and to determine intra-rater agreement). All clips and images were subsequently randomly ordered within the entire collection, giving the impression to the reader that each image represented a unique unrelated 'case'.

Echocardiographic analysis and AMVL assessment protocol

19 experienced cardiac sonographers were appointed as readers for this study. All sonographers were accredited with the British Society for Echocardiography (BSE) and formed part of the 2018 and 2019 Echo in Africa (EIA) volunteer groups. The data capture phase of the study took place in October 2018 where nine readers evaluated the cases. This process was repeated again in September 2019 where 10 different readers evaluated the same cases. On the day of the study, all readers attended a training session led by the lead investigator (LDH) detailing the WHF AMVL assessment. Further details of this tutorial and the WHF measurement protocol are included in the supplementary material-Addendum . All readers were blinded to the original study concept and design and had no knowledge that they were to evaluate subjects with a prior history of ARF.

The readers were randomly divided into two groups that read cases generated from two separate cohorts. To prevent reader fatigue, each group only reviewed eight unique studies, amounting to 128 consecutive 'cases'. To ensure that an optimal assessment of each 'case' was performed, a rest break of 30 minutes was scheduled after completion of 64 'cases'. Each reader was allocated to a Personal Computer (PC) that served as a private image reviewing station. Offline measurements were performed using a Digital Imaging and Communications in Medicine (DICOM) viewer (RadiANT™ Version 4.6.9, Poznan, Poland). The DICOM viewer has both a measurement and zoom function that allows for an accurate AMVL assessment.

Readers were requested either to self-select an optimal frame from the pre-selected cine-loop and perform an AMVL measurement (reader-optimised image) or perform a measurement based on an optimised still-image, preselected by the lead investigator (specialist-optimised image).The reader optimised the still-frame either with or without zoom according to a randomised, predetermined order. The readers were requested to perform the measurement in accordance with the WHF protocol (see supplementary material-Addendum). Measurements were made in millimetres to an accuracy of two decimal places and were manually entered into a separate data collection sheet. AMVL assessments were made independently by each reader who were blinded to each other's assessment. There was no time limit set for each 'case' assessment.

Statistical analyses

For descriptive statistics, the mean of the two repeat readings was calculated for each reader and case. The mean and standard deviation of the readings were calculated across the experimental settings and further expressed as a coefficient of variation(CV).[11] We estimated intraclass correlation coefficient (ICC) as measures for assessing the inter-rater and intra-rater reliability of AMVL readings based on the methodology proposed by Gwet.[12] This was done separately for the four experimental image settings and study cohort. Inter - and intra-rater ICCs were calculated using a 2-way random-effects model with readers, cases, random effects and the repeated readings of each reader. This was implemented in Stata version 15 using the kappaetc command.[13]

5.4. Results

Study population

A summary of the ARF cohort characteristics are included in Table 5. 1. The average age of patients evaluated in Cohort 1 was 15 years (range 9-22 years). The average time elapsed since diagnosis of ARF was four years and three months (range 1-15 years). According to the original medical documentation, six of the patients were identified with carditis at index-ARF diagnosis with the majority of cases (5/8) demonstrating current WHF RHD of the mitral valve ('definite-' and 'borderline RHD') at the time of enrolment. Cohort 2 was an older group with an average age of 21 years (range 10 – 37 years) and a longer period since ARF diagnosis of 11 years and 10 months (range 1-26 years). Seven of the patients were identified with carditis at the time of index ARF diagnosis with 5/8 cases demonstrating current WHF RHD of the MV.

Summary statistics from Cohort 1 and 2

The complete dataset by reader and case is included in the supplementary material (see Addendum B, Table i) with additional descriptive statistics from each respective cohort by case (see Addendum B, Table ii and reader (see Addendum B, Table iii).

Reliability analysis

The inter-rater agreement between readers in both cohorts was poor regardless of the underlying setting with an ICC ranging between 0.13-0.46 (Table 5. 2). Overall, the degree of agreement between readers evaluating Cohort 2 was higher than that in Cohort 1. The setting of specialist-optimised images without zoom was associated with the highest degree of agreement in both cohorts with an ICC of 0.29 (95%, CI 0.11-0.65) and 0.46 (95%, CI 0.23-0.79) respectively. This was followed by reader-optimised images without zoom [ICC 0.23(95%, CI 0.07-0.59) and 0.45 (95%, CI 0.23-0.78)]. The addition of zoom to specialist-optimised images decreased agreement further [ICC 0.20 (0.06-0.54) and 0.36 (0.15-0.72)]. The setting associated with the poorest agreement profile was that of reader-optimised images with zoom with an ICC of 0.13 (95%, CI 0.03-0.45) and 0.34 (95%, CI 0.15-0.70) respectively. The CV across all cases and measurement setting was high, ranging from 18-51%. (see Addendum B, **Error! Reference source not found.**) The intra-rater agreement between readers in both cohorts ranged from moderate to good across all settings with an ICC ranging between 0.64- 0.86. The difference in agreement profiles between each cohort and setting was negligible.

5.5. Discussion

We assessed the reliability of the current WHF AMVL thickness measurement. Whilst the measurement demonstrated a moderate degree of repeatability, the reproducibility is poor and deteriorates further with the use of an optimised, zoom-assisted measurement. Accordingly, based on our findings, we cannot endorse the current assessment as a reliable method for RHD identification.

Three notable findings are highlighted in our reliability study. The first is that an AMVL measurement was poorly reproducible(ICC<0.5) across all evaluated settings in both cohorts (Table 5. 2). Similarly, the AMVL measurement was imprecise in each evaluated case with a high level of dispersion (18-51%) demonstrated around the CV value(Addendum B, **Error! Reference source not found.**).

It is particularly notable that the degree of inter-rater agreement demonstrated amongst the specialised-optimised image setting remained poor. The specialist-optimised still-image setting was included to provide a hypothetical ‘reference standard’ with which to demonstrate the best possible agreement statistic for the AMVL assessment. A poor agreement amongst this setting would suggest that despite controlling for all sources of potential bias, the AMVL cannot be reliably measured due to an inherent, degree of variability that is introduced by the reader.

The second notable finding in our study relates to the role of zoom in an AMVL measurement. A mandatory zoom-optimised measurement was introduced into each setting with the premise that it could allow for a more accurate ‘edge-to-edge’ delineation by the reader and improve overall reliability. However, the addition of zoom resulted in a further reduction in inter-rater agreement when compared to the reference standard assessment. This seemingly paradoxical effect would appear to suggest that in practise, the zoom function should not be used to optimise the AMVL prior to measurement. Further study is required to determine the reasons underlying this finding. One

potential explanation may relate to the nature of zoom functionality which allows for magnification of an image without an increase in resolution. The consequence is that the reader is required to accurately identify the margins of the leaflet in an indistinct, pixelated image; thus contributing to a greater degree of variability.

The third notable finding from our study reflects the repeatability of the AMVL measurement within our cohort. Here, the intra-rater agreement was moderate-to-good (ICC 0.64-0.86) with a narrow confidence interval range. (Table 5. 2) Similarly, the degree of agreement was maintained across both settings (specialist-and reader optimised images with and without zoom). Given our findings, it would appear that each individual reader demonstrates an adequate propensity to consistently identify and measure what they believe to be the true AMVL. The problem however is that readers are unable to agree on what constitutes the AMVL leaflet (i.e. the true edge-to-edge measurement). This point highlights a major limitation in AMVL measurement; namely that the identification of the true leaflet is subject to a significant degree of inherent inter-rater variability. Our study findings reflect our anecdotal experience gained whilst screening high-risk children in the Western Cape, South Africa as part of the Echo in Africa project. We find the process of obtaining an accurate and reproducible AMVL measurement complex, labour intensive and, as in the case of two published RHD screening series; subject to a wider degree of inter-rater variability.[8,9]

The WHF AMVL measurement methodology

The current WHF guideline attempts to standardise this process by advocating a specific measurement methodology and requires that the echocardiographer/and or reader make a number of important decisions. However, due to the inherent anatomical complexity of the AMVL , a measurement protocol would struggle to address and standardise each AMVL measurement. We consider the measurement methodology to follow a so-called decision tree that is borne out in a number of steps and/or decisions with each having a potential influence the final AMVL measurement.

The first decision (provided the echocardiographic machine is set according to the WHF specifications i.e. probe using recommended frequency, optimised gain settings without harmonics), relates to the optimal capture of the mitral valve in a PSLAX cine-loop. The onus lies with the screener to decide which part of the mitral leaflet is sectioned. This may include performing a so-called ‘parasternal-sweep’ to look for focal rheumatic disease involving the medial or lateral aspects of the leaflet.[3,14] In the process a number of cine-loops are saved for subsequent evaluation. Invariably, more than one cine-loop is saved by the screener shifting the responsibility onto the reader to make a second decision to identify the loop most representative of the true AMVL.

This decision is particularly challenging as the current guideline does not state which section of the leaflet(central, medial or lateral) should be consistently assessed, stating rather that the thickest portion of the leaflet be identified. The thickest portion of the leaflet may not always represent the true leaflet, reflecting rather a composite of leaflet tissue and overlying strut chordae. This ‘composite

leaflet' is frequently encountered by the reader when evaluating the anterolateral or posteromedial portions of the AMVL where the insertion points of strut chordae predominate. In this case, it can be particularly challenging to confidently differentiate between the leaflet and chordal insertions in a healthy AMVL and those of a leaflet with focal rheumatic disease.

The third decision rests on the reader to stop and scroll through the cine-loop and identify the time point in the cardiac cycle where the AMVL is at full extension and free of overlying chordae, noting that these events are frequently not simultaneous requiring the reader to choose between time points.

The fourth decision requires the reader to confidently delineate the edge-to-edge border of the AMVL and perform a measurement. Here, over-measurement of the leaflet can easily occur as leaflet-chordal separation at the tips of the AMVL can be particularly difficult to achieve. This is often the area where early rheumatic disease is first evident, and it is critical to ensure that subvalvular tissue is not included in the measurement. An example of the complex interplay between some of these decisions is illustrated in Figure 5. 2.

The AMVL decision tree highlights some of the complexities that are encountered when evaluating and measuring an AMVL and delineates the extent to which a guideline can realistically address the degree of subjectivity inherent to an AMVL assessment. The agreement generated during this measurement process may also shed light on the discrepancies in the reliability statistics published in recent validation studies, specifically if we look closely at what decisions the readers in these studies were left to make.

Understanding published AMVL thickness measurement reliability statistics

Two recent reliability studies have demonstrated the AMVL measurement to be a repeatable and reproducible assessment with an interclass and intraclass correlation coefficient ranging between 0.75-0.85 and 0.79-0.90 respectively.[6,7] There are, however, significant methodological limitations in both of these studies that require further discussion.

The first study in question reports on the range of AMVL thickness amongst high-risk children in New Zealand and whose data has largely underpinned the WHF's endorsement of an AMVL measurement.[1,6] The reliability data was generated by two experienced readers who made three repeat measurements of the AMVL from an optimised still-image. It is unclear from the study methodology whether the selection and optimisation of the cine-loop was a reader- or study investigator-initiated process and whether the study instituted a randomisation process to safeguard against bias in their test-retest reliability analysis.

The second study evaluated the inter-and intra-rater reliability of the WHF criteria using an online platform that allowed readers to access and evaluate cases. For each AMVL assessment, the reader

was provided with a still-image of the AMVL in diastole with an attached measurement (provided by the study investigator) and was then required to repeat the same measurement. Both these validation studies have generated acceptable agreement statistics but only in the context of first removing most, if not all of the decisions outlined in the AMVL decision tree prior to the reader's measurement.

Although our study includes only a limited number of decisions required from each reader, it does support the hypothesis that increasing the numbers of decisions in the decision tree has a negative impact on overall reliability statistics.

Limitations

Our study methodology was designed to reduce the degree of bias in the test-retest analyses of an AMVL measurement. We addressed this issue by randomising each case and the assorted still-images and cine-loops throughout the study sequence. However, we cannot exclude the chance that the reader may have nevertheless recognised individual 'cases' and either recalled their initial measurement or at least remembered the point in diastole when the initial measurement was made. If this is the case, then the estimated inter- and intra-rater values may be an overestimation of the true reliability statistic.

The use of the acoustic zoom function (high resolution zoom) could have yielded images with improved temporal and spatial resolution and with it; a different set of reliability statistics. However the current handheld screening devices that are used in numerous large scale RHD screening campaigns around the world lack an acoustic zoom function. Our study setting findings thus remain relevant to our clinical contexts and in keeping with current practise.

Our study was powered to assess the reliability of the WHF AMVL measurement in a homogenous cohort of cases with a high likelihood of concurrent RHD. Thus, the size and nature of our study population limit the generalisability of our descriptive statistics. Further comprehensive method agreement analyses are required in larger studies that have evaluated high-risk heterogenous populations. These publications will be invaluable to guide the RHD community to provide definite proof of either an improved measurement strategy, or a de novo alternative method of RHD identification.

5.6. Conclusion

In response to various methodological limitations in studies validating the WHF AMVL assessment, we present a reliability analysis of the WHF AMVL measurement. Our findings demonstrate the AMVL measurement to be only moderately repeatable within readers and to have very poor reproducibility that is not improved by the addition of a zoom-optimised protocol. These findings call into question the reliability of the current WHF AMVL assessment for RHD. If these findings are confirmed it suggests

that more detailed analysis of the AMVL incorporating more than leaflet thickness alone is urgently required.

Acknowledgements

The Echo in Africa project is supported by an Edwards Lifesciences 'Every heartbeat matters' grant (#35526255).

5.7. References

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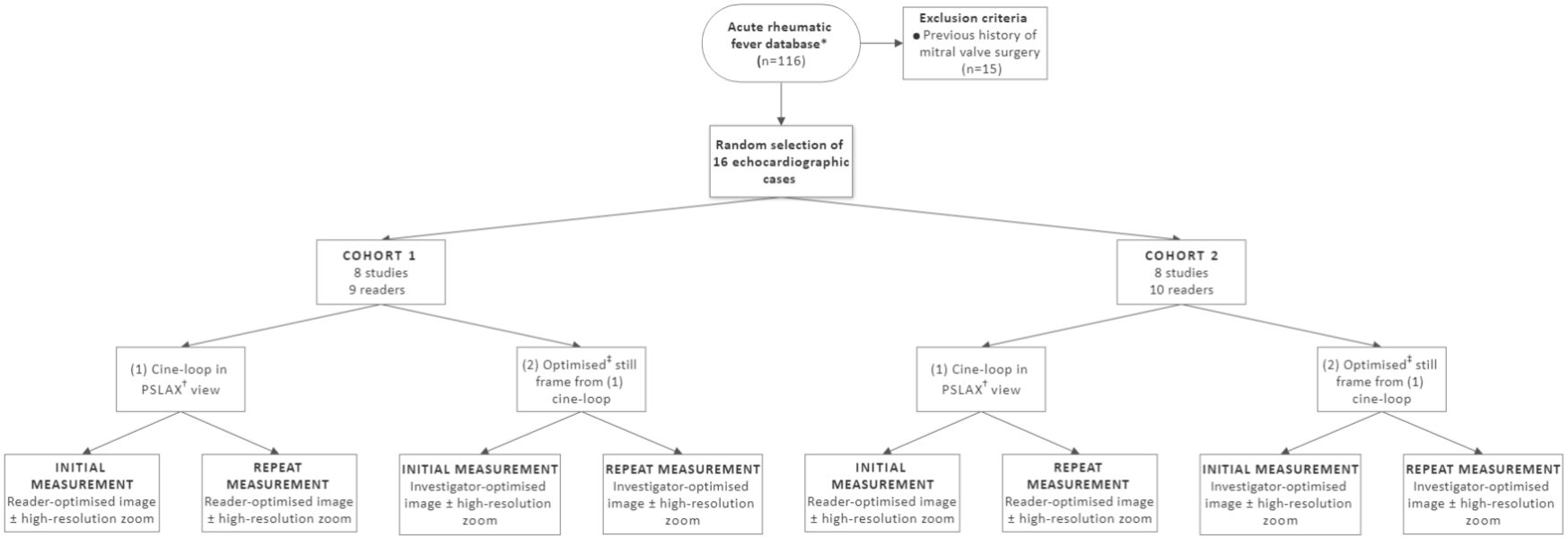
5.8. Figures

Figure 5. 1. Synopsis of study methodology

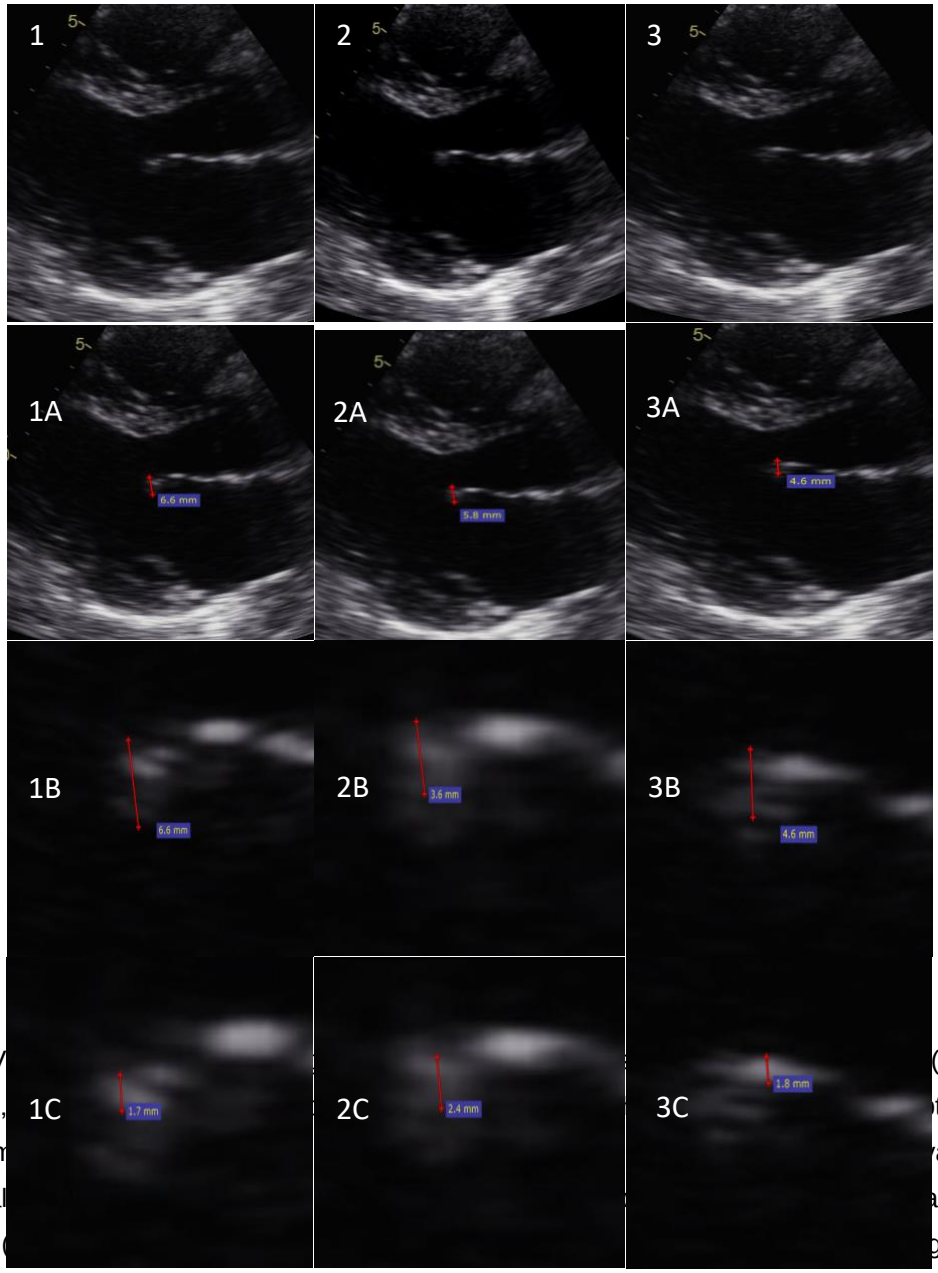


* Echocardiographic registry of patients with a prior documented history of acute rheumatic fever according to the Jones or Modified Jones criteria

† PSLAX, para-sternal long axis view

‡ Images were optimised by the specialist to ensure that the distal 1/3 section of the A2 segment of the AMVL was perpendicular to the ultrasound beam and maximally separated from overlying chordal tissue

Figure 5. 2. The complexity of an AMVL measurement and the impact of a zoom-optimised assessment on overall thickness



An AMV (<3mm) and abnormal (≥3mm), timise the measurement. The num valve leaflet (AMVL) at maximal tic fever (ARF). Still-images (g. Images 1A,2A,3A represent an edge-to-edge measurement of the AMVL (without zoom optimisation) with a measured range of 3.6-6.6mm. Images 1B,2B,3B are a zoom-optimised view of the measurement made in 1A,2A,3A and demonstrate that the initial assessment over-estimated the leaflet thickness. Images 1C,2C,3C represent a zoom-optimised measurement of the same images depicted in Image 1B,2B,3B and reflect a narrower range of measured thickness, falling within the WHF's ambit of normalcy (1.7-2.4mm).

5.9. Tables

Table 5. 1. Characteristics of selected echocardiography studies

Characteristic	Studies allocated to Group 1 (n=8)	Studies allocated to Group 2 (n=8)
Age (mean; range in years)	15; 9-22	21; 10-37
Time since index*RF diagnosis (mean; range in years)	4; 1-15	11; 1-26
†Carditis	6	7
‡WHF 'definite §RHD'	8	5

*rheumatic fever

†Clinical diagnosis based on the auscultation of a typical murmur indicating mitral and/or aortic regurgitation

‡World Heart federation

§rheumatic heart disease

Table 5. 2. Estimated inter-rater and intra-rater intraclass correlation coefficient (ICC) from the 2-way random-effects model using repeated readings

Setting	**Cohort 1**		**Cohort 2**	
	Inter-rater ICC (95%,CI)	**Intra-rater ICC (95%,CI)**	**Inter-rater ICC (95%,CI)**	**Intra-rater ICC (95%,CI)**
Specialist-optimised(no zoom)	0.29 (0.11-0.65)	0.77 (0.60-0.88)	0.46 (0.23-0.79)	0.78 (0.62-0.89)
Reader-optimised(no zoom)	0.23 (0.07-0.59)	0.81 (0.68-0.89)	0.45(0.23-0.78)	0.77 (0.61-0.89)
Specialist-optimised(zoom)	0.20 (0.06-0.54)	0.83 (0.70-0.91)	0.36 (0.15-0.72)	0.86 (0.76-0.93)
Reader-optimised(zoom)	0.13 (0.03-0.45)	0.78 (0.63-0.88)	0.34 (0.15-0.70)	0.64 (0.57-0.86)

5.10. Supplementary material

Addendum A

Description of tutorial and WHF measurement protocol

On the day of the study, all readers attended a 90-minute training session which was held in the Education Centre at the Division of Cardiology, Tygerberg Academic Hospital. They were informed that they were to take part in a study that evaluated the reproducibility of the anterior mitral valve leaflet (AMVL) measurement as described in the current World Heart Federation (WHF) criteria. During this session, the lead investigator (LDH) conveyed the WHF requirements for an accurate AMVL measurement. These are as follows:

- AMVL thickness should be measured during diastole at full excursion
- Measurement should be taken at the thickest portion of the leaflet, including focal thickening, beading, and nodularity
- Measurement should be performed on a frame with maximal separation of chordae from the leaflet tissue

Numerous cine-loops of pre-selected WHF ‘normal’ and ‘abnormal’ studies were presented for group review to consolidate their knowledge. In each of these examples, the lead investigator demonstrated how measurements can be optimised using the zoom function.

Addendum B

Table i

Complete dataset by reader, cohort and case

Cohort 1

Reader 1	Specialist-optimised image (no zoom)	Specialist-optimised image(2) (no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	1,2	2,2	1,6	1,5	1,9	1,7	1,9	1,6
Case 2	1	1,4	1,3	0,9	1,3	1,5	1,4	1,4
Case 3	2,1	2,3	1,4	1,4	2,2	1,8	1,8	2,1
Case 4	2,8	3,3	2,6	2,3	3,4	3,8	2,6	3,7
Case 5	2	2,4	2,2	1,6	3,6	3,7	2,2	2
Case 6	2,2	2	1,8	1,4	2,2	2,3	2,3	1,7
Case 7	3,1	1,6	1,3	1,3	1	1,5	1,4	1,3
Case 8	1,6	1,1	1,9	1,7	1,1	1,4	1,2	1,2

Reader 2	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	2,1	1,9	2,2	1,8	1,7	2,7	1,5	1,5
Case 2	2	1,7	1,3	1,6	2,1	2,5	1,7	1,6
Case 3	2,9	3,2	2,3	2,7	3	3,1	1,3	2,2
Case 4	3,2	3,1	3,2	3,3	3,2	2,9	2,1	3,3
Case 5	2,4	2,5	2,2	2,3	2,9	2,7	2,4	3,2
Case 6	2,2	2,2	2	2,1	2,8	2,7	1,5	3,3

Case 7	1,8	1,5	2	1,4	1,8	2,2	2,4	2,4
Case 8	1,6	1,9	1,6	1,8	2,3	2,3	2,4	2,3

Reader 3	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	1,8	1,1	1	1,1	1	1,2	1,1	1
Case 2	0,9	1	1,2	1	1,1	1	1,2	0,8
Case 3	2,1	2,2	1,5	2	1,4	2	1,1	2,7
Case 4	1,6	1,5	1,3	1,4	1,5	1,6	1,5	1,3
Case 5	2,1	1,3	1,6	1,3	2,3	1,8	1,3	1,3
Case 6	2	1,8	2	1,8	1,3	2	1,4	1,5
Case 7	1,3	1,3	1,4	1,3	1,3	1,5	1,5	1,2
Case 8	0,9	1,4	1,2	1	1,3	1,5	1,1	1,5

Reader 4	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	1,8	2,2	2	1,8	2,3	2	1,9	1,7
Case 2	1,1	1,5	1,3	1,3	2,6	1,5	2,1	2,7
Case 3	2,4	2,3	2	2	1,8	2,7	1,7	1,9
Case 4	2	2,1	1,8	1,5	2	3,4	2,1	2,6
Case 5	2,5	2,5	2,2	2,4	2,8	2,8	2,1	2,3
Case 6	2,2	2,2	2,2	1,8	2,6	2	1,5	2,2
Case 7	2,4	2,4	1,5	1,5	1,9	1,9	1,7	1,7

Case 8	1,7	1,6	1,5	1,2	1,7	2	1,6	1,3

Reader 5	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	2,3	2	2	1,9	2	2	1,7	2,2
Case 2	1,7	1,5	1,5	1,5	1,8	1,4	1,9	1,6
Case 3	2,6	2,4	2,7	3,2	2,6	2,5	2,7	2,6
Case 4	3,1	3,1	3,5	3,5	3,6	3,3	3,1	3,4
Case 5	3,1	2,2	2,6	2,4	3	2,8	2,8	3,1
Case 6	2,3	2	2,2	2,5	3,3	2,6	3	3,3
Case 7	2,2	1,7	1,9	2	2,6	2,2	2,6	2
Case 8	2,1	2,3	1,9	1,8	2,2	3,4	2,3	2

Reader 6	Specialist-optimised	Specialist-optimised	Specialist-optimised	Specialist-optimised	Reader-optimised	Reader-optimised	Reader-optimised	Reader-optimised

	image(no zoom)	image(2)(no zoom)	image(with zoom)	image(2)(with zoom)	image(no zoom)	image(2)(no zoom)	image(with zoom)	image(2)(with zoom)
Case 1	3	2,6	2,8	3,2	2,4	2,4	2,2	2,7
Case 2	2,6	2,7	2,8	2,8	3,2	2,7	2,8	3,6
Case 3	3,2	4,6	3,1	3,2	3,1	3	2,6	2,9
Case 4	3,6	3,5	2,7	2,8	4	2,9	3,1	3
Case 5	3,3	3,7	3,6	4,4	3,1	4	2,8	2,9
Case 6	3,3	3,7	3,9	3,6	2,8	2,7	2,7	2,5
Case 7	2,5	2,8	3,1	2,9	2,5	2,7	2,5	2,7
Case 8	1,8	3,1	2,1	3	3,2	1,8	2,7	3,2

Reader 7	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	3	3,1	2,9	2,9	2,5	2,8	2,2	2,5

Case 2	2,6	1,5	1,3	1,5	1,9	1,5	1,5	1,9
Case 3	4,1	4,2	4,1	4,2	3,8	4,2	4,3	4
Case 4	4,7	4	4,1	4,2	4,4	4,7	4,6	4,6
Case 5	2,5	2,9	2,4	2,9	2,6	2,5	3,2	3,1
Case 6	4,2	3,5	2,5	4,2	4,6	4,2	3,8	5,4
Case 7	1,6	2,4	1,6	2,2	2,3	2,1	1,8	2,5
Case 8	1,9	2,9	2,2	3,3	2,8	2,9	2,6	2,7

Reader 8	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	1,9	2,3	1,7	1,4	2	1,5	1,9	1,3
Case 2	2,3	2,3	2,5	2,3	3,5	3,3	3,3	3,1
Case 3	3,1	2,7	2,5	2,4	2,5	2,7	2,3	2,2
Case 4	2,8	2,8	3,3	2,3	2,5	2,3	2,1	2,3
Case 5	2	2,3	1,9	2,1	2,1	2,4	1,9	2,4
Case 6	2,3	2,6	1,9	2,3	2,8	2,8	2,3	2,3
Case 7	2	2,1	1,6	1,9	1,5	2,2	1,8	1,8
Case 8	3,1	1,7	2,2	1,7	1,8	2,2	1,8	1,9

Reader 9	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)

Case 1	1,6	1,8	1,5	1,4	1,7	2,1	0,8	1,6
Case 2	1,1	1,1	1,2	1,3	1,3	1,6	1,4	1,3
Case 3	2,4	2,3	1,3	2	1,5	2,1	1,4	1,4
Case 4	3,3	2,3	1,5	3,7	2,8	3	1,6	3,1
Case 5	2,3	1,9	1,9	2,2	2,5	1,6	1,8	2,2
Case 6	1,3	1,6	1,7	1,8	1,9	2,3	1,8	1,5
Case 7	0,9	1,4	1,2	1,2	1,3	1,5	1,3	1,4
Case 8	1,2	2,5	1,4	1,8	2,9	3,1	1,8	1,9

Table i

Complete dataset by reader, cohort and case

Cohort 2

Reader 10	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 9	1,5	1,9	1,8	0,6	1	0,9	1	1,1
Case 10	1,7	1,4	1,5	1,3	2,2	0,8	1,4	1,5
Case	1,4	1,6	1,2	1,1	1,2	0,7	0,8	0,8

11								
Case 12	1	0,8	0,7	0,7	3,4	1,5	2,3	2,2
Case 13	1,1	3,6	0,8	2,6	2	0,9	1,3	1,1
Case 14	5	5,5	3,8	2,8	4,7	4,9	3,8	3,6
Case 15	1	1,3	0,7	0,8	1,3	2,3	1,8	1,7
Case 16	1	0,6	0,7	0,7	1,2	0,8	0,7	2,3

Reader 11	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 9	0,8	3,8	3,9	3,3	1,7	3,9	1,2	4,2
Case 10	2,4	2,7	2,2	2,1	2,2	2,5	1,2	1,8
Case 11	2	1,8	2,1	1	1,1	2,7	1,3	1,5
Case 12	1,6	1,8	1,3	1	3,1	4,3	2,4	3
Case 13	1,8	1,9	1,2	1,6	1,8	1,7	1	1,4
Case 14	3,8	4,4	4,3	4,1	4,6	3,8	5	4,1
Case	2,8	1,6	1,3	1,3	2	2,5	3	2

15								
Case 16	1,5	1,2	0,8	0,7	1,7	1,7	1,6	1,6

Reader 12	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 9	1,8	3,7	2,5	2,1	2,7	4,5	1,5	1,7
Case 10	1,9	2,3	1,6	2,5	2,8	2,6	1	1,4
Case 11	2,6	2,2	1,4	1,5	1,6	1,9	1,5	1,5
Case 12	4,1	4,5	2,7	3,9	3,3	4,1	3,2	3,9
Case 13	1,7	2,3	1,6	2,3	2,3	1,7	1,8	3,1
Case 14	3,3	4,4	3,4	3,5	3,9	3,5	2,4	4,5
Case 15	1,8	3,1	3	1,9	2	1,8	2,6	3,2
Case 16	1	1,5	0,9	1,3	1,2	1,7	1,4	1,6

Reader	Specialist-	Specialist-	Specialist-	Specialist-	Reader-	Reader-	Reader-	Reader-

13	optimised image(no zoom)	optimised image(2)(no zoom)	optimised image(with zoom)	optimised image(2)(with zoom)	optimised image(no zoom)	optimised image(2)(no zoom)	optimised image(with zoom)	optimised image(2)(with zoom)
Case 9	2,7	2	1,6	1,2	2,5	1,9	1,4	2,1
Case 10	1,8	1,9	1,8	1,5	1,9	2,3	1,2	1,8
Case 11	1,8	2,2	1,6	1,5	2,1	2,1	2	1,3
Case 12	2,2	2,3	1,8	2,7	3,5	3,3	2,8	2,9
Case 13	1,5	1,5	1,6	1,4	1,8	2,3	1,9	2,1
Case 14	2,9	3,2	2,2	1,4	3,1	3,5	2,9	2,3
Case 15	3	2,4	1,9	2	2,4	1,8	1,7	1,7
Case 16	1,4	1,5	1,1	0,9	1,7	1,8	1,6	1,1

Reader 14	Specialist-optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 9	2,4	2,4	1,5	1,5	2,7	2,4	1,4	1,5
Case 10	2,3	2,5	1,5	1,8	2,4	2,2	1,5	1,9
Case 11	1,9	1,8	1,1	1,4	1,6	2,2	1	1,3
Case 12	3,1	3,1	2,2	2,7	3,1	3,5	2,3	3,2

Case 13	1,7	2	0,6	1,5	2,2	2,2	1,5	1,2
Case 14	3,9	4,6	3,2	3,8	4,3	4,2	2,9	3,9
Case 15	2,5	2,4	0,8	1,3	2,1	2	1,8	1,6
Case 16	1,6	1,3	0,8	0,7	1,8	1,9	1,1	1,4

Reader 15	Specialist - optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	1,7	2,2	2,1	1,7	1,9	2,4	2	1,6
Case 2	1,3	1,4	1,6	1,7	1,5	1,4	1,7	1,3
Case 3	1,3	2,2	1,2	1	1,5	1,4	1,1	1,4
Case 4	1,6	2,1	1,9	1,6	3,1	2,5	2,3	1,9
Case 5	1,3	2,3	1,4	2	1,8	1,2	1,5	1
Case 6	2,7	3,3	2,3	2,1	3,3	2,7	4,2	2,3
Case 7	1,8	2,7	1,6	1,8	1,4	2,1	1,2	1,7
Case 8	1	1,3	1,1	1	1,5	1,7	1,3	1,7

Reader 16	Specialist - optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	2,5	2,5	2	2,6	3,2	3,2	3,5	3,5
Case 2	1,8	1	1	1,1	2,6	2,7	3,3	2,7

Case 3	1,4	1,3	1,2	1,5	1,3	0,9	0,9	1,3
Case 4	2,3	2,2	2,7	1,3	3,1	2,3	3,3	3,1
Case 5	1,3	1,3	1,9	1,4	1	1,4	1,1	1
Case 6	3,5	3,3	3,9	3,9	2,7	1,6	2,9	2,6
Case 7	3,5	3,2	2,9	2,5	2	1,8	1,6	3
Case 8	1,3	0,8	2,5	1	0,9	1,4	1,1	2,6

Reader 17	Specialist - optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	3	3	3,1	3,2	5,6	4,5	1,5	2,2
Case 2	1,9	2,2	1,7	2,2	2,7	2,8	3,6	2,5
Case 3	2,7	2,1	2,3	2,8	2,7	3	2,5	3
Case 4	4,7	4,3	3	4,1	3,5	3	2,8	4
Case 5	1,8	1,7	1,1	1,6	1,2	2,5	1,7	4,9
Case 6	3,8	3,8	3,7	3,8	3,7	4	3,8	4
Case 7	2,9	2,7	3,2	3,4	1,8	2	3	3,1
Case 8	2,4	0,9	1	1,1	1,5	1,7	1,9	1,7

Reader 18	Specialist -	Specialist-optimised	Specialist-optimised	Specialist-optimised	Reader-optimised	Reader-optimised	Reader-optimised	Reader-optimised

	optimised image(no zoom)	image(2)(no zoom)	image(with zoom)	image(2)(with zoom)	image(no zoom)	image(2)(no zoom)	image(with zoom)	image(2)(with zoom)
Case 1	4,4	3,9	4	3,9	5,5	3,5	4	4
Case 2	2,3	2,5	2,7	2,6	2,5	2,3	3,5	3,2
Case 3	2,1	2,6	3	2,8	2,6	2,3	3,9	3,2
Case 4	4	4	4	4	4,9	4,4	4,8	4
Case 5	2,5	2,2	2,2	2,2	2,5	2,8	2,4	2,2
Case 6	5,1	4,5	5,1	5	4,2	4	5,8	4
Case 7	3,9	3,2	3,5	3,5	2,6	2,8	2,8	3
Case 8	2,6	1,6	1,6	1,6	1,8	1,8	1,4	1,8

Reader 19	Specialist - optimised image(no zoom)	Specialist-optimised image(2)(no zoom)	Specialist-optimised image(with zoom)	Specialist-optimised image(2)(with zoom)	Reader-optimised image(no zoom)	Reader-optimised image(2)(no zoom)	Reader-optimised image(with zoom)	Reader-optimised image(2)(with zoom)
Case 1	4,2	3,5	3,7	4,2	4,9	3	5,9	3,9
Case 2	2,9	2,9	2,6	2,6	3,1	3,2	2,6	3,2
Case 3	3,4	2,9	2	2,3	2,2	3,9	2,6	3,2
Case 4	3,5	3,5	2,7	3,2	3,9	3,5	3,4	3,6
Case 5	2,7	2,7	3,9	2,3	2,9	3,1	3,1	3,6
Case 6	5	4,4	4,3	5	5,6	4,7	5,7	5,7
Case 7	4,8	3,5	3,6	3,9	3,8	3,3	4	3,6
Case 8	1,6	3,3	2,9	3	3,1	3,3	2,4	2,3

Table ii

Descriptive statistics by case for reader agreement

Cohort 1 Case 1	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	9	9	9	9
	Mean	2.10	1.92	1.99	1.73
	SD	0.51	0.65	0.44	0.47
	Min	1.45	1.05	1.10	1.05
	Max	3.05	3	2.65	2.45
	CV	0.24	0.33	0.22	0.27

Cohort 1 Case 2	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	9	9	9	9
	Mean	1.66	1.58	1.98	1.96
	SD	0.58	0.59	0.77	0.79
	Min	0.95	1.10	1.05	1.00
	Max	2.65	2.80	3.40	3.20
	CV	0.35	0.37	0.38	0.40

Cohort 1 Case 3	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	9	9	9	9
	Mean	2.83	2.44	2.55	2.28
	SD	0.73	0.87	0.73	0.82
	Min	2.15	1.40	1.70	1.40
	Max	4.15	4.15	4.00	4.15
	CV	0.26	0.35	0.28	0.35

Cohort 1 Case 4	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	9	9	9	9
	Mean	2.93	2.72	3.07	2.78
	SD	0.80	0.86	0.84	0.89
	Min	1.55	1.35	1.55	1.40
	Max	4.35	4.15	4.55	4.60
	CV	0.27	0.31	0.27	0.32

Cohort 1 Case 5	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	9	9	9	9
	Mean	2.43	2.34	2.73	2.38
	SD	0.50	0.71	0.58	0.59
	Min	1.70	1.45	2.05	1.30
	Max	3.50	4.00	3.65	3.15
	CV	0.20	0.30	0.21	0.24

Cohort 1 Case 6	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	9	9	9	9
	Mean	2.42	2.31	2.66	2.44
	SD	0.76	0.73	0.77	0.95
	Min	1.45	1.60	1.65	1.45
	Max	3.85	3.75	4.40	4.60
	CV	0.31	0.31	0.29	0.39

Cohort 1 Case 7	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)

	N	9	9	9	9
	Mean	1.94	1.73	1.88	1.88
	SD	0.50	0.54	0.46	0.48
	Min	1.15	1.20	1.25	1.35
	Max	2.65	3.00	2.60	2.60
	CV	0.25	0.31	0.24	0.25

Cohort 1 Case 8	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	9	9	9	9
	Mean	1.91	1.86	2.21	1.97
	SD	0.48	0.50	0.63	0.60
	Min	1.15	1.10	1.25	1.20
	Max	2.45	2.75	3.00	2.95
	CV	0.25	0.27	0.28	0.30

Cohort 2 Case 9	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10
	Mean	2.69	2.56	3.09	2.46
	SD	0.78	1.02	1.22	1.27
	Min	1.70	1.20	0.95	1.05
	Max	4.15	3.95	5.05	4.90
	CV	0.28	0.40	0.39	0.51

Cohort 2 Case 10	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10
	Mean	2.05	1.88	2.33	2.11
	SD	0.51	0.50	0.53	0.84
	Min	1.35	1.05	1.45	1.20
	Max	2.90	2.65	3.15	3.35
	CV	0.25	0.26	0.23	0.39

Cohort 2 Case 11	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10
	Mean	2.06	1.68	1.95	1.80
	SD	0.52	0.63	0.69	0.92
	Min	1.35	1.10	0.98	0.80
	Max	3.15	2.90	3.05	3.55
	CV	0.25	0.37	0.35	0.51

Cohort 2 Case 12	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10
	Mean	2.83	2.34	3.30	3.07
	SD	1.22	1.07	0.63	0.68
	Min	0.90	0.70	2.45	2.10

	Max	4.50	4.00	4.65	4.40
	CV	0.43	0.46	0.18	0.22

Cohort 2 Case 13	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10
	Mean	1.94	1.82	1.96	1.94
	SD	0.42	0.51	0.55	0.87
	Min	1.30	1.35	1.20	1.05
	Max	2.70	3.10	3.00	3.35
	CV	0.26	0.28	0.28	0.45

Cohort 2 Case 14	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10
	Mean	4.02	3.45	3.85	3.82
	SD	0.75	1.05	0.87	0.97
	Min	3.00	1.80	2.15	2.60
	Max	5.25	5.05	5.15	5.70
	CV	0.18	0.31	0.22	0.25

Cohort 2 Case 15	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10
	Mean	2.70	2.31	2.19	2.40
	SD	0.83	0.99	0.55	0.76
	Min	1.15	0.75	1.75	1.45
	Max	4.15	3.75	3.55	3.80
	CV	0.30	0.43	0.25	0.31

Cohort 2 Case 16	Variable	Specialist-optimised (no-zoom)	Specialist-optimised (zoom)	Reader optimised (no-zoom)	Reader-optimised (zoom)
	N	10	10	10	10

	Mean	1.47	1.30	1.71	1.63
	SD	0.49	0.60	0.59	0.31
	Min	0.80	0.70	1.00	1.25
	Max	2.45	2.95	3.20	2.55
	CV	0.33	0.51	0.34	0.19

Table iii

Descriptive statistics for the readers and cases of each cohort using the average of the two repeated measurements

Cohort 1 (cases 1-8 and readers 1-9)

Reader	Variable	Specialist-optimised (no zoom)	Specialist-optimised (with zoom)	Reader-optimised (No zoom)	Reader-optimised (with zoom)
1	N	8	8	8	8
	Mean	2.28	2.31	2.58	2.51
	SD	0.45	0.65	0.58	0.56
	Min	1.60	1.50	1.60	1.75
	Max	3.1	3.5	3.45	3.25
2	N	8	8	8	8
	Mean	3.12	3.10	2.90	2.80
	SD	0.54	0.50	0.42	0.25
	Min	2.45	2.55	2.40	1.75
	Max	3.90	4	3.55	3.20
3	N	8	8	8	8
	Mean	3.06	2.90	3.11	1.34
	SD	0.93	0.97	1.06	1.14
	Min	2	1.40	1.70	2.45
	Max	4.35	4.15	4.55	4.60
4	N	8	8	8	8
	Mean	2.39	2.12	2.38	2.16
	SD	0.31	0.40	0.54	0.48
	Min	2.05	1.55	1.75	1.7
	Max	2.90	2.80	3.40	3.20
5	N	8	8	8	8
	Mean	1.82	1.69	2.08	1.64
	SD	0.59	0.45	0.59	0.39
	Min	1.10	1.20	1.40	1.60

	Max	2.80	2.60	3	2.35
6	N	8	8	8	8
	Mean	2.01	1.63	2.15	1.86
	SD	0.59	0.41	0.97	0.61
	Min	1.20	1.10	1.25	1.20
	Max	3.05	2.45	3.65	3.15
7	N	8	8	8	8
	Mean	2.26	2.11	2.55	2.19
	SD	0.57	0.56	0.40	0.49
	Min	1.65	1.45	2	1.50
	Max	3.15	3.25	3.05	2.8
8	N	8	8	8	8
	Mean	1.51	1.38	1.48	1.34
	SD	0.39	0.31	0.32	0.27
	Min	0.95	1.05	1.05	1
	Max	2.15	1.90	2.05	1.90
9	N	8	8	8	8
	Mean	2.05	1.76	2.25	1.94
	SD	0.40	0.33	0.34	0.32
	Min	1.30	1.30	1.85	1.45
	Max	2.50	2.30	2.80	2.40

Table iii

Cohort 2 (cases 9-16 and readers 1-10)

Descriptive statistics for the readers and cases of each cohort using the average of the two repeated measurement

Reader	Variable	Specialist-optimised (No zoom)	Specialist-optimised (with zoom)	Reader-optimised (no zoom)	Reader-optimised (with zoom)
1	N	8	8	8	8
	Mean	1.88	1.63	1.96	1.76
	SD	0.56	0.38	0.62	0.66
	Min	1.15	1.05	1.45	1.25
	Max	3.00	2.20	3.00	3.25
2	N	8	8	8	8
	Mean	2.07	2.08	2.00	2.34
	SD	0.94	0.89	0.80	0.93
	Min	1.05	1.05	1.10	1.05
	Max	3.40	3.90	3.20	3.50
3	N	8	8	8	8
	Mean	2.74	2.58	2.88	2.88
	SD	1.00	1.02	1.16	0.93
	Min	1.65	1.05	1.60	1.05
	Max	4.50	3.75	5.05	3.50
4	N	8	8	8	8
	Mean	3.21	3.23	3.15	3.37
	SD	1.03	1.11	1.08	1.09
	Min	2.10	1.60	1.80	1.60
	Max	4.80	5.05	4.65	4.90
5	N	8	8	8	8
	Mean	3.40	3.26	3.59	3.67
	SD	0.77	0.80	0.71	1.11
	Min	2.45	2.15	3.00	2.35
	Max	4.70	4.65	5.15	5.70
6	N	8	8	8	8
	Mean	1.90	1.36	1.86	1.71
	SD	1.44	0.86	1.29	0.91
	Min	0.80	0.70	0.95	0.80
	Max	5.25	3.30	4.80	3.70
7	N	8	8	8	8
	Mean	2.24	2.01	2.58	2.26
	SD	0.83	1.23	0.92	1.10
	Min	1.35	0.75	1.70	1.20

	Max	4.10	4.20	4.20	4.55
8	N	8	8	8	8
	Mean	2.63	2.25	2.60	2.26
	SD	0.99	0.81	0.95	0.94
	Min	1.25	1.10	1.45	1.20
	Max	4.3	3.45	3.70	3.55
9	N	8	8	8	8
	Mean	2.14	1.63	2.37	1.92
	SD	0.55	0.37	0.61	0.53
	Min	1.45	1.00	1.75	1.35
	Max	3.05	2.25	3.40	2.85
10	N	8	8	8	8
	Mean	2.46	1.63	2.55	1.84
	SD	0.87	0.38	0.82	0.80
	Min	1.45	1.05	1.85	1.15
	Max	4.25	2.20	4.25	3.40

Chapter 6: The variable spectrum of anterior mitral valve leaflet restriction in rheumatic heart disease screening

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6.1. Abstract

Introduction

Anterior mitral valve leaflet (AMVL) restriction is a prominent morphological feature of rheumatic heart disease (RHD). Despite this, there is no strict definition of AMVL restriction in the current World Heart Federation (WHF) screening guideline. In the Echo in Africa (EIA) project, we define AMVL restriction when the tip of the leaflet points away from the interventricular septum towards the posterior left ventricular wall during peak diastole in the parasternal long-axis (PSLAX) view. 'Screen-positive' restriction cases demonstrate two distinct leaflet configurations. The first displays 'distal tip' AMVL restriction and typically is associated with additional morphological features of RHD. The second displays 'gradual bowing' AMVL restriction; an 'arch-like' leaflet configuration extending from the base to tip of the AMVL. Typically, the latter case involves the medial portion of the leaflet and have no associated features of RHD. We hypothesise that this configuration is a normal variant and unrelated to RHD. This study aims to determine the prevalence and associated leaflet configurations of AMVL restriction observed in schoolchildren with an established ' very low'-(VLP), 'high'-(HP) and 'very-high'-prevalence (VHP) of RHD.

Methodology

Three separate cohorts of EIA-screened children were identified based on their pre-assessment risk and post-test established rate of WHF 'definite RHD'. The first analysis determined the prevalence of 'gradual bowing'- and 'distal tip'-AMVL restriction amongst the VLP and HP cohort. The second analyses determined the prevalence of 'gradual bowing-' and 'distal tip'-AMVL restriction in a VHP cohort of WHF 'definite RHD' cases affecting the mitral valve (MV). To address concerns of incorporation bias, the assessment of AMVL restriction was removed from the WHF diagnostic schema in each of the VHP cases and only cases that still met WHF 'definite RHD' were evaluated.

Results

In the first analysis, 936 studies were evaluated (HP 577 cases; VLP 359 cases). Sixty-five cases of 'gradual bowing' AMVL restriction were identified in the HP cohort (11.3%, 95%, CI 8.9-14.1) and 35 cases(9.7%, 95%, CI 7-13.2) in the VLP cohort (p=0.47). The medial portion of the AMVL was affected in the majority of cases in the HP (58/65; 89.2%) and VLP cohorts (25/35; 71.4%). No cases with isolated 'gradual bowing' AMVL restriction in the HP- or VLP- cohort had associated morphological features of RHD. Two cases of 'distal tip' AMVL restriction were identified in the HP cohort and one case in the VLP cohort. In the second analyses, 43 studies were evaluated. 'Distal tip' AMVL restriction was identified in all 43 VHP cases (100%) and affected the central portion of the AMVL in all cases. There was a statistically significant difference in the prevalence of 'distal tip' AMVL restriction between the VLP and VHP cohort (p<0.0001).

Conclusion

AMVL restriction is a common finding amongst school children screened for RHD. Further classification of restricted leaflet morphology highlights 'gradual bowing' AMVL restriction as a common finding and a normal, benign variant of the MV. In comparison, 'distal tip' AMVL restriction was present in all cases in the VHP cohort and was localised to at least the central portion of the leaflet in all cases. No cases of WHF 'definite RHD' of the MV in this analysis exhibited a straight, non-restricted central portion of the AMVL. This novel finding requires further investigation and prospective evaluation to test its validity as a potential predictive screening tool to rule out RHD of the MV.

6.2. Introduction

Restricted anterior mitral valve leaflet (AMVL) motion is recognised by the current World Heart Federation (WHF) screening criteria as a prominent morphological feature of rheumatic heart disease (RHD).[1] In established rheumatic valvular lesions such as mitral stenosis (MS), the pattern of leaflet tip restriction, valvular thickening, and characteristic diastolic 'doming' configuration of a relatively mobile mid-leaflet is considered pathognomonic for RHD (Media clip 6. 1). Here, descriptive terms such as 'hockey-stick' -, 'dog's leg'- and 'elbow'-deformity aptly

characterise the echocardiographic features of an AMVL affected by advanced rheumatic disease. However, the extent to which these colloquial terms reliably discern early rheumatic-related restriction of the AMVL is unclear.

All things considered; leaflet restriction in its purest form relates to the lack of normal leaflet separation when the valve opens. In rheumatic mitral valve disease (MVD), leaflet restriction is typically confined to the leaflet tips until late in the disease with the mid and basal portions remaining pliable with normal separation. The resultant effect on the leaflet configuration is diastolic doming (or bowing) of the leaflet with the tip pointing 'downward' towards the posterior left ventricular wall and away from the interventricular septum at maximal leaflet separation (Figure 6. 1).

Under the auspices of the Echo in Africa (EIA) project (large-scale RHD screening project in the Western Cape, South Africa), we have developed a strict screening definition of AMVL restriction. We recognise AMVL restriction when the tip of the leaflet points away from the interventricular septum towards the posterior left ventricular (LV) wall during peak diastole in the parasternal long-axis (PSLAX) view. Using this definition, we can identify subtle, rheumatic-related, so-called 'distal tip' AMVL restriction (Media clip 6. 2). The designation of 'distal tip' AMVL restriction highlights the fact that the basal and mid leaflet typically appears straight or linear at peak diastolic leaflet extension with an abrupt downward angulation of the leaflet tip, typically seen anywhere from the distal third of the leaflet towards the tip. However, anecdotal evidence from the EIA experience has highlighted another distinct AMVL phenotype with 'screen-positive' restriction that deserves further study. The configuration of the AMVL in these cases is 'arch-like' and primarily affects the medial portion of the leaflet.

Media clip 6. 3 and Media clip 6. 4). In these cases the arch-like bowing of the leaflet typically affects the entire length of the leaflet from the basal portion to the tip, giving rise to a more gradual but recognizable arch. Given the absence of additional morphological features of RHD to explain the degree of leaflet restriction, we hypothesise that this leaflet pattern (as we will now refer to as 'gradual bowing' AMVL restriction) should be considered a normal variant. The primary objective of this study is to describe the prevalence, and associated leaflet configuration of AMVL restriction observed in three cohorts of South African school children with an established 'very-high'-, 'high'-and ' very-low'- prevalence of RHD.

6.3. Methods

Study design, setting and participants

Two separate retrospective analyses of EIA screening data were performed to investigate the prevalence and associated leaflet configuration of AMVL restriction.

AMVL restriction in a high-and low-RHD prevalence cohort

The first analyses evaluated EIA screening data captured from two secondary schools situated in the Western Cape, South Africa. Each school was designated as either 'high' RHD- (HP) or 'very low' RHD-prevalence (VLP)

based on their established rate of WHF 'definite RHD' .[2] (see Chapter 3; Inter-scallop separations of the posterior mitral valve leaflet: a solution to the 'borderline RHD' conundrum?).

The HP cohort was a state (public) school situated in Cape Town, a region with a high prevalence of RHD.[3] Briefly, 577 school children (mean age 15.5, range 13-18 years) were screened, of whom, 348 (60.3%) were female. Five cases of WHF 'definite RHD' (8.7 cases per 1000 [95% CI, 3.7-20.3]) and 25 cases of WHF 'borderline RHD'(43 cases per 1000 [95% CI,29.5-63.2] were identified in this cohort.

The VLP cohort was an independent (private) school, situated in the Cape Winelands district. There were 359 school children (mean age 15.5, range 13-18 years) screened in this cohort, of whom, 189 (52.6%) were female. There were no cases of WHF 'definite RHD' identified in this cohort. Ethical approval for the study was obtained through the University of Stellenbosch (S17/02/030) and the relevant governing bodies of each school. Parental/guardian consent was required before study enrolment.

AMVL restriction in a 'very-high' RHD prevalence cohort

The established rate of WHF 'definite RHD' cases in the HP cohort (five cases) still represents low absolute numbers that would preclude us from making any definitive statements regarding the morphology of true rheumatic AMVL restriction. To better describe the pattern of restriction seen in cases with true rheumatic MVD, we studied a third cohort of cases with a 'very-high' RHD prevalence (VHP). This VHP cohort was generated by including all WHF 'definite RHD' cases affecting the MV (i.e. WHF definite subcategories A, B and D)[1] that were identified by the EIA project between 2014-2019. Importantly, to address the risk of incorporation bias (where the index test comprises part of the gold standard), the assessment of AMVL restriction was removed from the WHF diagnostic schema in each of the VHP cases and only cases that still met WHF 'definite RHD' were evaluated (i.e. two morphological features [excluding AMVL restriction]) and a 'pathological' regurgitant jet).

Sample size calculation

High-and very low-RHD prevalence cohort

The prevalence of AMVL restriction with a 'gradual bowing' configuration has not been determined in RHD screening echocardiography. For a sample size calculation, we assumed a prevalence of 50% (worst case scenario for estimation). Accordingly, a sample size of 384 subjects from each cohort would accurately determine the prevalence of AMVL restriction with 5% precision with 95% confidence. The sample size of 384 in each cohort would have 80% power to detect at least a 10% difference in the prevalence of AMVL restriction between the cohorts at a 5% significance level. The actual enrolled sample size of the very low-prevalence cohort (359 participants) was determined to have a minimal impact on the power of the study.

'Very high'- RHD prevalence cohort

According to current echocardiographic screening data in HP communities, the expected case distribution of WHF 'definite RHD' is 1% with a documented prevalence of leaflet restriction in WHF 'definite RHD' of ~95%.[4] We expected a sample size of 50 such cases over the 5 years. For a 50% prevalence of AMVL restriction in this expected sample of n=50, the precision will be 13.4% with 95% confidence.

Screening procedure

All enrolled participants underwent an initial screening study with a handheld (HH) device (GE Medical Systems, Milwaukee, Wisconsin, USA) using a 1.7- to 3.8MHz transducer probe (G3S). Children with an 'abnormal' screening study (mitral regurgitation jet length ≥1.5cm, aortic regurgitation jet length ≥ 0.5 cm or any morphological features of RHD) underwent a validation study with a standard portable machine (GE Vivid I, Milwaukee, USA) with a 2- to 3.6 MHz transducer probe (GE 3S). A single operator (LDH) performed all the echocardiographic assessments. Both studies were captured according to a detailed standardised protocol that has been described elsewhere (see Chapter 3; Inter-scallop separations of the posterior mitral valve leaflet: a solution to the 'borderline RHD' conundrum?).

Briefly, both protocols included a comprehensive analysis of the MV in the PSLAX view. A parasternal sweep (screening technique described by EIA)[5] was used in each case to capture a single-beat cine-loop of the central (A2), medial(A3) and the lateral (A1) portions of the AMVL.

Data analysis

Using our screening experience gained in EIA, we defined AMVL restriction into one of four subcategories before the initial data analysis. The first subcategory was reserved for cases with 'gradual bowing' AMVL restriction. These cases demonstrate an 'arch-like' leaflet configuration extending from the base to tip of the AMVL (Figure 6.). The second subcategory was reserved for AMVL cases with 'distal tip' AMVL restriction. Here, the basal- to mid-leaflet shape was linear while the distal tip was restricted, pointing to the posterior LV wall at peak diastole, resulting in an abrupt, discrete transition between the mid- and distal 1/3 of the AMVL ( ). A third subcategory was reserved for cases with 'mixed' leaflet configurations, i.e. both 'distal tip'- and 'gradual bowing'-AMVL restriction identified in separate regions of the AMVL. A fourth subcategory accounted for indeterminate cases that did not fulfil the definition provided in any of the first three groups. The location of maximal AMVL restriction in all cases was classified according to the mitral valve segmentation model proposed by Carpentier.[6] Briefly; the AMVL is divided into three separate segments typically corresponding to the three more clearly defined PMVL scallops. Roughly, this results in the lateral third of the AMVL designated as 'A1', the central third of the leaflet as 'A2' and the medial third as 'A3'.

Reliability analysis (intra-and inter-observer variability)

A non-probabilistic sampling methodology was used to attain a case distribution ideal for the evaluation of the reliability of our restriction definition and associated leaflet configurations with a kappa statistic calculation. Firstly, a 10%, random sample of study participants was selected from both HP and VLP cohorts. Secondly, this sample was augmented with all the cases of 'gradual bowing' AMVL restriction that had not been randomly sampled and

with all the cases collected in the 'modified' WHF (VHP) cohort. All studies were anonymised, randomised and re-read independently by the primary investigator (LDH) and a second expert reader (PGH). The lead investigator and the reader were required to note: a) the presence of AMVL restriction, and b) the underlying leaflet configuration as previously described.

Statistical analysis

Data were entered into an Excel 2019 database (Microsoft), and statistical analysis was conducted in Stata 15 (StataCorp 2017). The prevalence of AMVL restriction and each predefined leaflet configuration was estimated with 95% confidence intervals (CIs) using the Wilson approach. The prevalence and leaflet configuration of AMVL restriction was compared between the cohorts using Fisher's exact test. A two-sided p-value of less than 0.05 was considered to indicate statistical significance. For the determination of inter-observer agreement, the lead investigator and the reader were required to note the presence of AMVL restriction and whether the leaflet morphology was consistent with a 'distal tip'- or 'gradual bowing'-AMVL configuration. The interpretation of kappa values was based on the Landis and Koch guidelines.[7] The proportion of agreement was reported as mean percentages with a 95% confidence interval (CI) for inter-rater agreement.

6.4. Results

In the first analyses, a total of 936 screening echocardiograms were evaluated for the presence of AMVL restriction. All cases with AMVL restriction could be further classified into predefined subcategories based on the observed leaflet morphology.

Restricted AMVL's with a 'gradual bowing' leaflet configuration was equally prevalent and accounted for the majority of cases in both the HP (65/577 cases, 11.3%) and VLP cohorts (35/359 cases, 9.7%; p=0.47 , Table 6. 1).The medial portion of the AMVL was affected in the majority of cases in the HP (58/65; 89.2%) and VLP cohorts (25/35; 71.4%). No cases with 'gradual bowing' AMVL restriction in either the VLP or HP cohort had additional WHF morphological features of MV disease including AMVL thickening or posterior mitral valve leaflet (PMVL) restriction.

Two cases of 'distal tip' AMVL restriction were identified in the HP cohort and one case in the VLP cohort. Both cases with 'distal tip' AMVL restriction in the HP cohort were 'screen-positive' for WHF 'definite RHD'. The position of 'distal tip' AMVL restriction was isolated to the central (A2) segment in one case and was generalised (A1-A3) in one case. In both HP cases, thickening of the distal AMVL and PMVL restriction were present. In the VLP case with 'distal tip' AMVL restriction, the position of restriction was central (A2) with no AMVL tip thickening or PMVL restriction. There were no cases of AMVL restriction with a mixed or indeterminate leaflet configuration in either cohort.

In the second analyses, 43 cases with 'modified' WHF 'definite RHD' of the MV were evaluated and constituted the VHP cohort. AMVL restriction with a 'distal tip' configuration was identified in all cases, representing a statistically significant finding when compared to the VLP cohort($p<0.0001$). Associated AMVL tip thickening was present in all cases in the VHP cohort and PMVL restriction was present in 36/43 cases (83.7%). The position of 'distal tip' AMVL restriction affected at least the central portion of the AMVL in all cases (Table 6. 2). No cases of isolated 'gradual bowing' AMVL restriction were identified in this cohort. A 'mixed' leaflet configuration was identified in 2/43 cases (4.7 %), both of which demonstrated a 'gradual bowing' configuration that was confined to the medial portion (A3) of the AMVL (Table 6. 2).

Assessment of Interobserver Agreement

The agreement between readers on the presence of AMVL restriction was substantial (κ=0.69; 95% CI, 0.58-0.80) with a proportion of agreement of 88%. The interobserver agreement in identifying 'gradual bowing' AMVL restriction configuration was substantial (κ=0.74; 95%CI, 0.65-0.83) with a percentage agreement of 87.5%. There was almost perfect agreement between readers on the identification of 'distal tip' AMVL restriction (κ=0.86; 95% CI, 0.77-0.95) with a proportion of agreement of 96%.

6.5. Discussion

This study proposes a novel definition for the detection of AMVL restriction in echocardiographic RHD screening. Furthermore, it investigates the prevalence of two morphologically distinct patterns of AMVL restriction encountered in a large-scale RHD screening program in South Africa. The results of this study highlight 'gradual bowing' AMVL restriction as a probable benign, normal variant of the MV. This pattern demonstrated a predilection for affecting the medial segment (A3) of the leaflet and was strongly associated with normal AMVL tip thickness. In comparison, 'distal tip' AMVL restriction was tightly correlated with the rheumatic process and affected the central segment (A2) of the AMVL in all VHP cases. Leaflet tip thickening and PMVL restriction were both significantly correlated with this pattern. An interesting finding from the study is that no cases of WHF 'definite RHD' of the MV in either cohort exhibited a straight, non-restricted central portion of the AMVL. This novel finding requires further investigation and prospective evaluation to test its validity as a potential predictive screening tool to rule out RHD of the MV. Overall, our findings highlight the importance of a strict, screening definition of AMVL restriction within an RHD screening algorithm. This, in conjunction with an appreciation for the variable leaflet morphologies of AMVL restriction and their relative morphological and rheumatic associations, could improve the delineation between RHD-related AMVL restriction and non-RHD-related AMVL restriction.

The 2012 WHF guideline was the first screening algorithm to recognise AMVL restriction as a prominent, specific morphological feature of latent RHD. However, the guideline does not offer a strict screening definition of AMVL restriction. Instead, colloquial terms such as 'hockey-stick' -, 'dog's leg-' and 'elbow'- deformity are used that typically characterise the AMVL configuration seen in cases with advanced disease where echocardiographic findings are overt and additional leaflet tip thickening coexists (i.e. mitral stenosis). In our experience screening

high-risk children as part of the Echo in Africa (EIA) project, we have found that the absence of a strict, reproducible definition of AMVL restriction negatively affects the application of this criterion for two reasons. Firstly, the degree of valvular restriction and thickening when present in the screening setting (including the EIA project) is often mild or very mild when compared to valves with clinically significant dysfunction where these terms were originally derived from. More subtle restriction in the screening setting may therefore not conform to these gross colloquial descriptions and may therefore be missed. Secondly, the currently used colloquial descriptions do not clearly discriminate between apparently different forms of AMVL restriction that appear to have very different associations with pathology. These reasons may go a long way to explain the findings of a recent study that evaluated the reproducibility of the WHF criteria. Here, WHF MV restriction, together with chordal thickening, was identified as one of the least reproducible WHF morphological criteria (Kappa 0.55, 95%, CI 0.49-0.60).[8]

In the present study, we sought to address these potential limitations within the WHF guideline by introducing a strict screening definition of AMVL restriction. Using this definition, we found an equal proportion and relatively high number of AMVL restriction cases in both the HP (67 cases, 11.6%) and VLP cohorts (35 cases, 9.7%). The high numbers alone (particularly in the VLP cohort) already hint to a non-rheumatic aetiology for the bulk of this restriction given the much lower known rate of RHD (borderline and definite) in multiple published high-risk RHD screening cohorts (2.5%-4.1%).[4,9,10] Very low-risk cohorts (such as the VLP cohort in this study) would be expected to have essentially no true RHD cases making high prevalence findings such as ‘gradual bowing’ AMVL restriction unlikely to be related to RHD. In this study, we recognised two distinct leaflet morphologies associated with AMVL restriction. The first was characterised by a ‘gradual bowing’ AMVL configuration and was a frequent finding affecting both risk cohorts equally (9.8 and 11.3% in HP and VLP cohorts respectively [p=0.47] ; Table 6. 1). Similarly, the position of ‘gradual bowing’ AMVL restriction was predominantly isolated to the medial segment of the AMVL (A3) in both cohorts, accounting for the majority of cases (Table 6. 1) and, as expected for a non-rheumatic aetiology, was not associated with other WHF morphological features of RHD.
The underlying mechanism of ‘gradual bowing’ AMVL restriction remains unclear, and in particular its predilection for the medial segment of the AMVL. Further work is required to deepen our understanding of the valve dynamics underlying this observation. Nevertheless, our findings support the initial hypothesis that ‘gradual bowing’ AMVL restriction is likely to represent a normal variant and if found in its typical medial position, should not be ascribed to underlying RHD.

The second leaflet morphology was characterised by ‘distal tip’ AMVL restriction which in the first analysis was an uncommon finding (two HP cases with WHF ‘definite disease’ and a single VLP case that had no associated features suggestive of RHD). The second analysis of the study evaluated a VHP group that consisted of screened cases with WHF ‘definite RHD’ affecting the MV. This cohort was included with a view to describing rheumatic restriction in the screening context and to identify whether it differed from the normal variant of restriction identified in the very low-risk cohort. Here, an important observation was made; ‘distal tip’ AMVL restriction was identified in all VHP cases and involved the central portion of the valve (A2) in all 43 cases (100%; Table 6. 2).

Therefore, in our study, no cases of WHF 'definite RHD' of the MV exhibited a straight, non-restricted central portion of the AMVL. This tight association between 'distal tip' restriction affecting the central portion of the leaflet and WHF 'definite' RHD of the MV should prompt further study to determine the utility of this as a possible RHD 'rule-out' screening tool. Based on the data from this study, the absence of 'distal tip' restriction in the central segment of the AMVL would exclude a case as having WHF 'definite RHD' of the MV. An accurate 'rule-out' screening tool has the potential to improve screening efficiency and significantly reduce screening times. If this is coupled with a low false-positive rate, the requirement of re-reads by a specialist would be significantly reduced. The number of central MV restriction cases (of any morphology) in the VLP cohort in the current study was low (1.6%, 6/359)) supporting the possible utility of this criterion for rule-out screening. AMVL restriction in the central portion of the leaflet as a screening criterion has the advantage of being a non-measurement -based means of MV assessment lending itself to evaluation with cheaper HH devices. The criterion is relatively simple to apply and requires acquisition in a single PSLAX view only. This culminates in a short overall study time and may raise the possibly of upskilling and task shifting healthcare workers to become adept in basic screening that deliver school-based RHD screening in underserved regions.

The study findings underscore the importance of recognising and appreciating 'distal tip' AMVL restriction as a prominent morphological feature of 'latent' RHD amongst our South African cohort. As this is the first study to test an alternative, independent screening definition of AMVL restriction (i.e. independent of the morphological changes observed in the PMVL), we are unable to confidently compare our findings with those reported by other large-scale screening programs situated in other parts of the world. Moreover, not all screening studies publish the individual WHF criteria that constitute their WHF 'definite RHD' cohort. Despite this, we can infer from published screening literature that 'restricted leaflet motion' using the current WHF definition is not the most consistent finding identifying WHF 'definite RHD' of the MV. Here, we note published data from the Programa de rastreamento da valvopatia reumática (PROVAR) study, recently used as a derivation cohort by Nunes et al. to develop a simplified echocardiographic score applicable for RHD screening with potential to predict disease progression.[11] Interestingly, thickening of the AMVL and excessive leaflet motion represented the two most frequent changes associated with WHF 'definite RHD' of the MV in their cohort. In contrast, restricted MV leaflet motion accounted for only 13 (22%) cases. Whether our findings of a tight association of 'distal tip' AMVL restriction (using a novel definition) with WHF 'definite RHD' represent a phenotype of MVD that is specific to our region or a useful, novel screening tool that could be incorporated into a screening guideline for use in underserved communities further abroad, remains to be seen. At present, the findings of this study suggest that in the context of screening for latent RHD, the absence of 'distal tip' restriction in the central portion of the leaflet would be a potentially strong predictor of no underlying rheumatic MVD. Ongoing prospective study, together with further collaborative work between large-scale screening programs, is required to gauge the significance of our study results.

Limitations

This study is a retrospective, single-centre observational study of a cohort of screened cases identified with AMVL restriction. As such, the fact that cases with AMVL restriction could be further subcategorised into two leaflet phenotypes that appeared to track normality versus RHD pathology is a finding that requires further prospective evaluation in a well-designed longitudinal study.

6.6. Conclusion

In this study, we describe a novel screening definition of AMVL restriction in RHD screening. We were able to identified two distinct morphological patterns of AMVL restriction; a 'gradual bowing' configuration that predominantly affecting the medial MV and appears to be a normal variant as well as a 'distal tip' AMVL restriction configuration with a significant association with RHD. The fact that 'distal tip' restriction was seen to affect the central portion of the AMVL in all cases within our VHP cohort and the fact that central leaflet bowing infrequently affected the VLP cohort is notable. This novel finding requires further investigation and prospective evaluation to test its validity as a potential predictive screening tool to rule out RHD of the MV.

6.7. References

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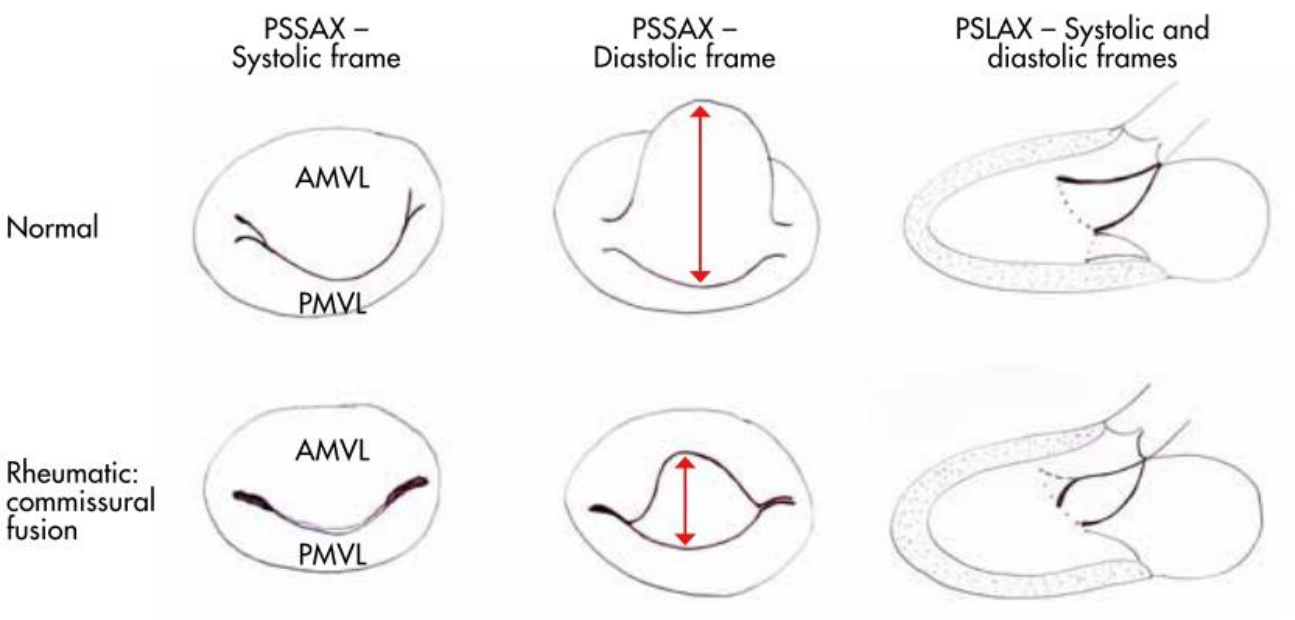
6.8. Figures

Figure 6. 1. Mechanism of AMVL restriction from commissural fusion of the mitral valve



The underlying pathogenesis of valvular restriction in established RHD is well described. It is related to varying degrees of chordal shortening, chordal fusion, leaflet thickening with shortening of total leaflet edge length, calcification and commissural fusion.[12] In this figure, the role of commissural fusion is described. Here commissural fusion limits vertical leaflet edge separation in diastole (see red arrow above). If AMVL length remains unchanged, this translates into restriction of the mobile leaflet body as seen in the PSLAX frames. The PSLAX images illustrate the fact that the leaflet tip motion is halted along the normal arc of motion. However, the body and or base continues to move forward leading to leaflet restriction and a 'downwards' facing leaflet tip which now points towards the posterior ventricular wall and away from the septum at maximal leaflet separation. PSSAX, parasternal short axis; PSLAX, parasternal long axis; AMVL, anterior mitral valve leaflet; PMVL, posterior mitral valve leaflet (Image reproduced with permission from PG Herbst)[27]

Figure 6. 2. Parasternal long-axis view of a screened case with 'gradual bowing' anterior mitral valve leaflet restriction.

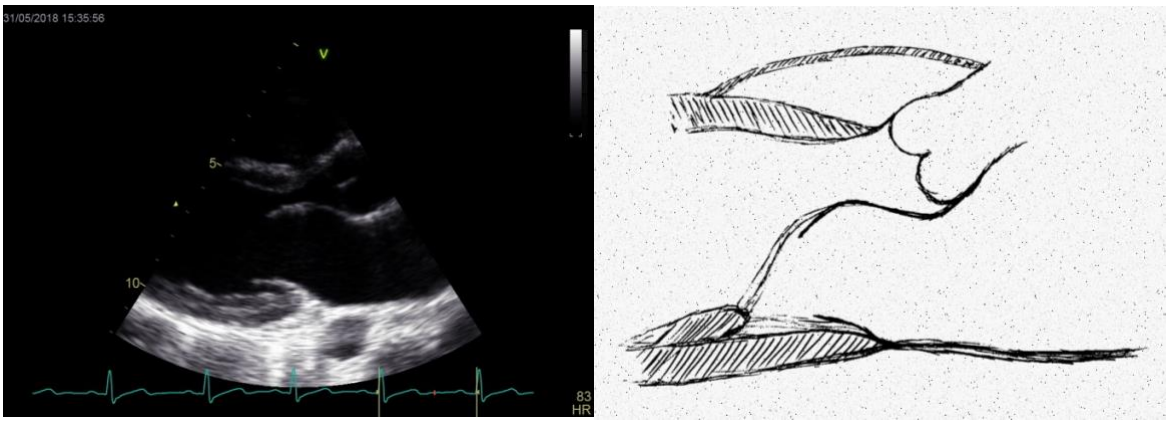


Figure 6. 3. Parasternal long-axis view of a screened case with ‘distal tip’ anterior mitral valve restriction.

6.9. Tables

Table 6. 1. Prevalence and location of AMVL restriction with 'gradual bowing' configuration

	High prevalence cohort (n=577)	**Low prevalence cohort (n=359)**
'Gradual bowing' AMVL restriction (n, %)	65(11.3) *	35(9.7) *
Location of AMVL restriction (n, %†) ‡		
Generalised (A1-A3)	2(3)	6(17.2)
Central (A2)	5(7.7)	4(11.4)
Medial (A3) n, %	51(78.5)	23(65.7)
Medial and central(A2-A3)	7(10.8)	2(5.7)

*Calculated as a percentage of the respective cohort

† Calculated as a percentage of 'gradual bowing' AMVL restriction cases in each respective cohort

‡No cases of restriction were noted in the medial and lateral (A3 +A1), lateral and central (A1-A2) and lateral (A1) portions of the AMVL

AMVL, anterior mitral valve leaflet

Table 6. 2. Prevalence and location of 'distal tip' AMVL restriction in a 'very-high' RHD prevalence cohort with WHF 'definite RHD'

***WHF 'definite RHD' cohort with AMVL restriction** (n=43)	**Location of AMVL restriction**				**Total** (n/%)
	Generalised (A1-A3)	Central (A2)	Medial (A3)	Medial and central (A2-A3)	
'Distal tip' AMVL restriction (n/%)	3(7)	36(83.7)	0(0)	2(4.6)	41(95.3)
'Gradual bowing' AMVL restriction (n/%)	0(0)	0(0)	0(0)	0(0)	0()
'Mixed' configuration AMVL restriction† (n/%)	0(0)	'Distal tip' 2(4.7)	'Gradual bowing' 2(4.7)	0(0)	2(4.7)

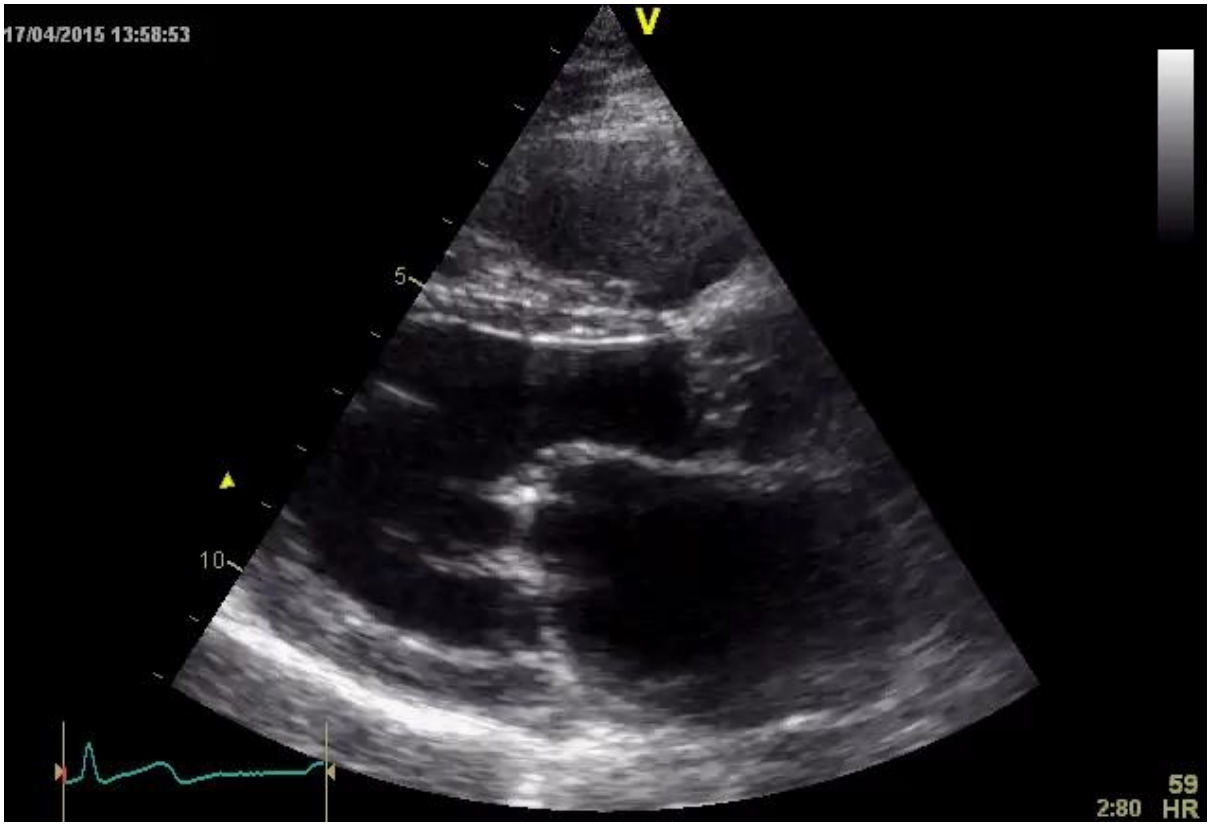
*Cases that still met diagnostic criteria for WHF 'definite RHD' despite the removal of AMVL restriction from the diagnostic schema

† Cases with both 'distal tip' AMVL- and 'gradual bowing'- AMVL restriction

AMVL, anterior mitral valve leaflet; RHD, rheumatic heart disease

6.10. Media clips

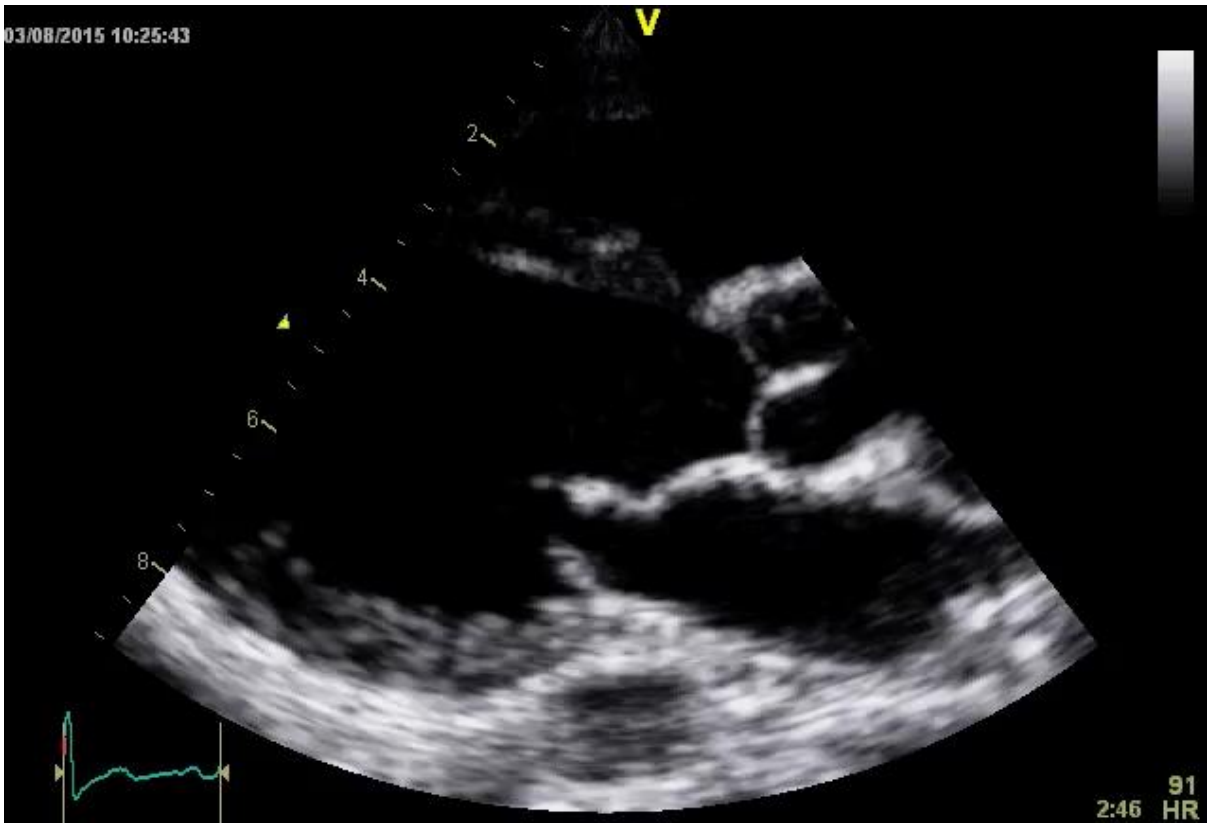
Media clip 6. 1. Screening 2D echocardiogram in the parasternal long-axis view of a case with mitral stenosis.



The anterior mitral valve leaflet (AMVL) tip is restricted, thickened and is ‘doming’ during diastole. Descriptive terms such as ‘hockey stick’, ‘dog’s leg’ and ‘elbow deformity’ are used to describe this pathognomonic pattern of RHD.

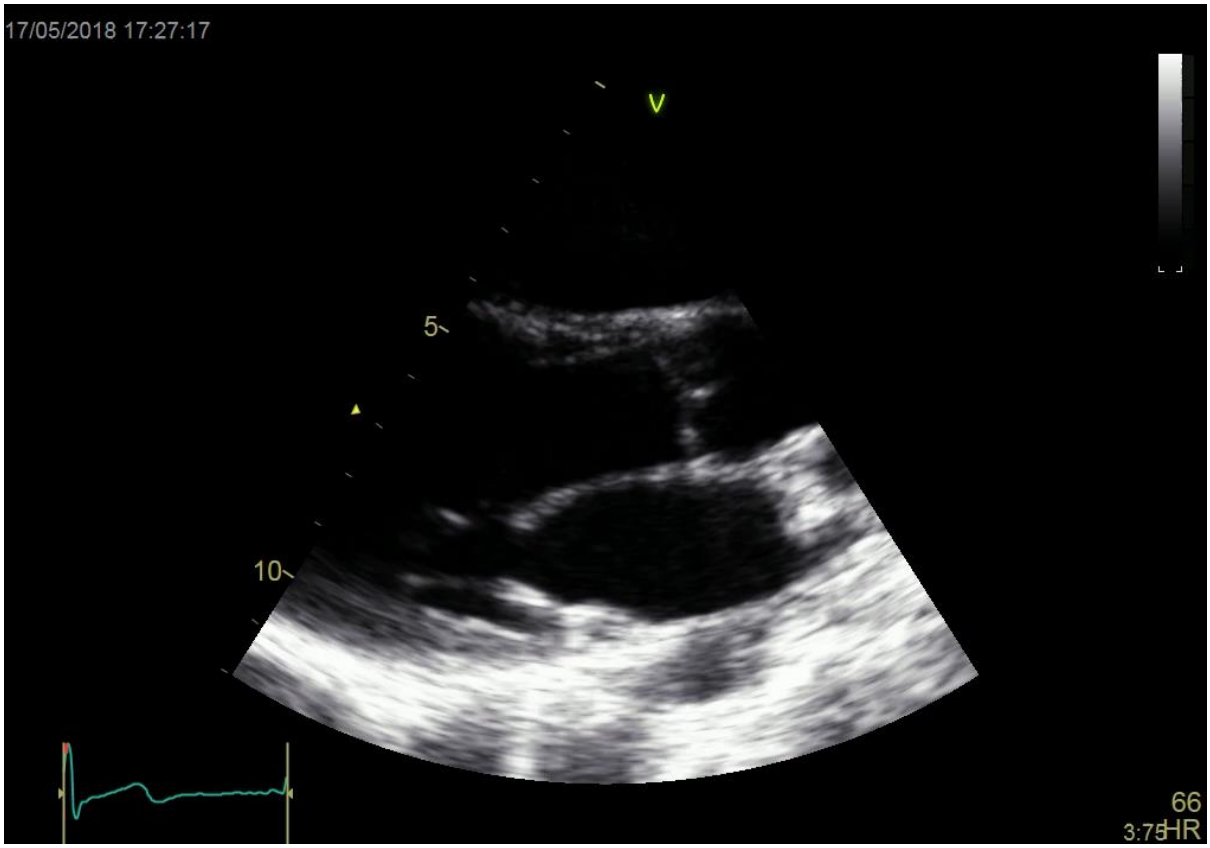
2D, two-dimensional; RHD, rheumatic heart disease

Media clip 6. 2. Screening 2D echocardiogram in the parasternal long-axis view of a case with RHD.



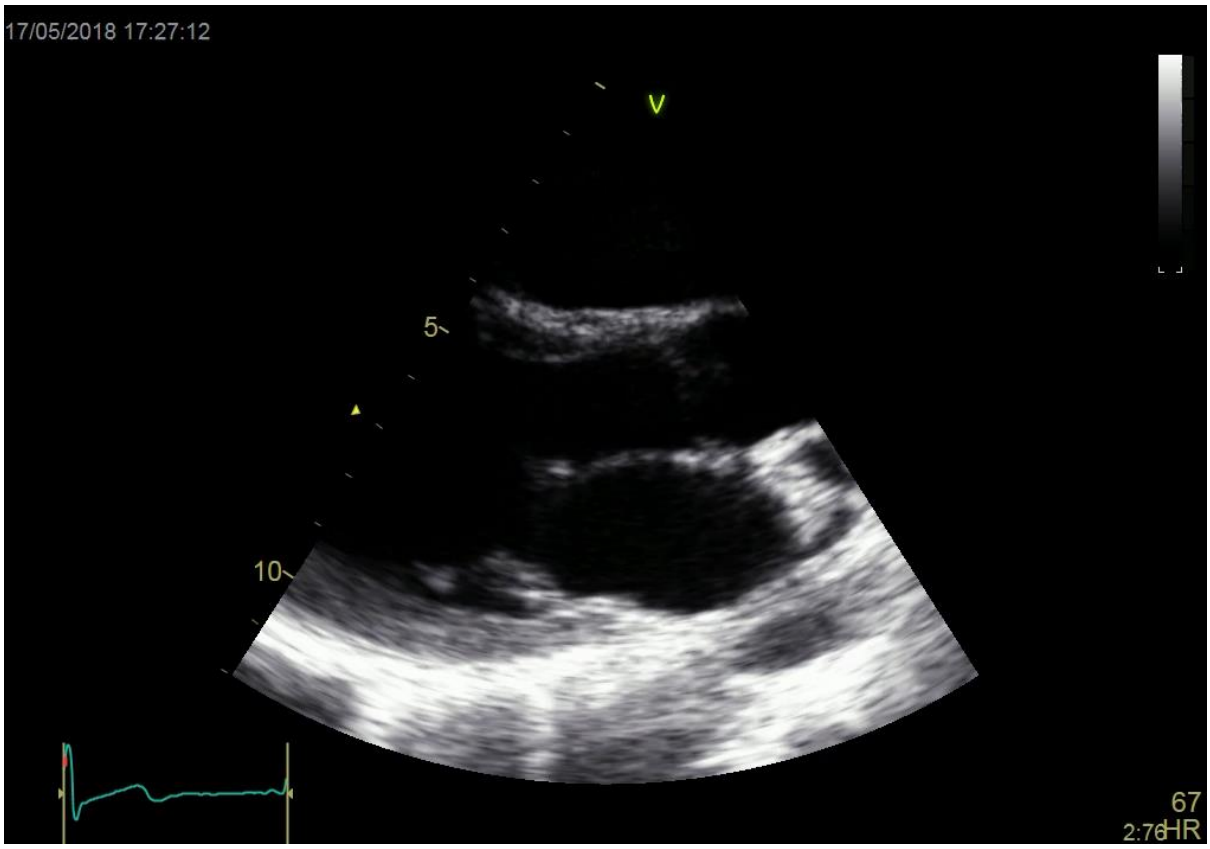
The tip of the AMVL is seen to point away from the interventricular septum towards the posterior left ventricular (LV) wall during peak diastole with a configuration typical of 'distal tip' AMVL restriction. Furthermore, there is thickening of the distal tip of the AMVL with restriction of the posterior mitral valve leaflet (PMVL). Further colour Doppler interrogation of the valve demonstrated 'pathological' mitral regurgitation(MR) with an underlying mechanism consistent with 'pseudoprolapse' of the AMVL[27] (not shown).

Media clip 6. 3. Screening 2D echocardiogram in the parasternal long-axis view of a typical case with anterior mitral valve leaflet restriction with a 'gradual bowing' configuration.



The leaflet restriction is localised to the medial aspect of the leaflet with no additional morphological features of RHD. A parasternal sweep is used to visualise the medial (tilt downwards) and lateral (tilt upwards) portions of the mitral valve.

Media clip 6. 4. Screening 2D echocardiogram in the parasternal long-axis view of the case presented in Media clip 6.3**.**



The echocardiographic probe has been tilted back to a ‘neutral position’ to visualise the central portion of the leaflet (A2). Here, there is no evidence of AMVL restriction.

Chapter 7: Morpho-mechanistic screening criteria for the echocardiographic detection of rheumatic heart disease

Chapter seven is a submission-ready manuscript that presents a novel set of morpho-mechanistic (MM) screening criteria for the diagnosis of RHD. The performance of the MM criteria is compared to the current WHF criteria by applying both to a very-low risk screened population and a gold standard RHD cohort. My role in the study included developing the study protocol and performing and capturing all echocardiographic assessments of all enrolled study participants. I am the primary author of the manuscript included in this chapter. The statistical analyses was performed with guidance from Dr CJ Lombard (Stellenbosch University, Division of Epidemiology and Biostatistics). I am the primary author of this manuscript included in this chapter. AJK Pecoraro, MJ Monaghan and GW Lloyd reviewed the final manuscript.
AF Doubell and PG Herbst were the co-supervisor and supervisor respectively. They supervised the study design and execution. Both reviewed the final draft of the manuscript.

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7.1. Abstract

Introduction
The feasibility and utility of early echocardiographic case-detection of latent RHD are doubtful given that the current World Heart Federation (WHF) screening criteria identify a significant proportion of false positive cases with WHF 'borderline RHD'. Based on our Echo in Africa experience (large-scale RHD screening project in South

Africa), we have developed a novel set of screening criteria that principally evaluate leaflet morphology and mechanism of regurgitation; the morpho-mechanistic (MM) diagnostic RHD screening criteria. In addition, we created an abbreviated MM RHD 'rule-out' test aimed at improving screening efficacy in the field. The current study evaluates the performance of the MM screening criteria and the MM RHD 'rule-out' test alongside the WHF criteria in a very low RHD-risk cohort where the prevalence of RHD is assumed to be negligible (i.e. a gold standard RHD-negative cohort defined to be true negative cases and used to estimate the false-positive rate) and in a very high-risk cohort with proven prior ARF and associated valvular dysfunction where the prevalence of RHD is deemed sufficiently high to define all cases as true RHD positive cases (i.e. a gold standard RHD-positive cohort used to estimate the false-negative rate).

Methods

The performance of both sets of screening criteria and the 'rule-out' test were retrospectively assessed in two pre-existing cohorts of study participants representing a gold standard RHD-negative cohort and a gold standard RHD-positive RHD cohort (prior, proven history of acute rheumatic fever and current evidence of valvular dysfunction, defined as MR≥2cm or AR≥1cm).

Results

The comprehensive echocardiograms of the gold standard RHD-negative cohort (n=364) and the gold standard RHD-positive cohort (n=65) were evaluated. In the gold standard RHD-negative cohort , the prevalence of MM 'borderline RHD' was 2.7 cases per 1000 population (95% CI,0.5-15.4) compared to the WHF 'borderline RHD' prevalence of 41.8 cases/1000 (95% CI,25.5-67.8; P<0.0004). Overall, the screening specificity of the MM- and WHF-criteria was 99.7% and 95.9% respectively. The MM RHD 'rule-out' test excluded 359 cases (98.6%).Assessment of the MM and WHF criteria in the gold standard RHD- positive cohort allowed for the assessment of an overall screening sensitivity for the detection of definite RHD of 92.4% and 89.2% respectively. The MM RHD 'rule-out' test did not exclude a single case from the gold standard RHD-positive cohort.

Conclusion

The MM criteria significantly reduced the false-positive rate of a borderline diagnosis in a gold standard RHD-negative cohort whilst maintaining a similar screening sensitivity compared to the WHF criteria within a gold standard RHD-positive cohort. Similarly, the MM RHD 'rule-out' test performed well by excluding the majority of cases within the gold standard RHD-negative whilst including all cases within the gold standard positive-RHD cohort, holding promise for the development of a two-step RHD screening algorithm to enable task shifting in RHD endemic regions. Further prospective study is required to investigate the feasibility of this approach and importantly whether the MM criteria identify those at highest risk for an unfavourable outcome.

7.2. Introduction

Screening echocardiography, guided by the current World Heart Federation (WHF) criteria, is recognised as the diagnostic investigation of choice for the detection of 'latent' RHD in asymptomatic, high-risk children.[1] The early detection and initiation of secondary prophylaxis in affected individuals remain attractive primary health care interventions, particularly in endemic regions with no or limited access to specialist cardiac services. However, the feasibility and utility of early case-detection are doubtful given that the majority of WHF-detected lesions (particularly those in the borderline category) apparently remain stable or even 'regress' in up to 88.8% of cases.[2] This somewhat paradoxical finding is, in our experience, related to the low specificity (high false-positive rate) of the current WHF criteria, where a large proportion of normal, non-rheumatic cases with isolated WHF 'pathological' MR are misclassified with borderline disease (see Chapter 3; Inter-scallop separations of the posterior mitral valve leaflet: a potential solution to the 'borderline RHD' conundrum?). The MR in these cases originates from slit-like separations between the scallops of the PMVL (so-called inter-scallop separations [ISS]) which we have described as a benign, normal variant of the PMVL.[3] To ensure further progress in this field, priority must be given to investigating whether an alternate screening approach could identify and exclude normal cases within the borderline group (i.e. increase specificity) while maintaining a high sensitivity for the detection of true RHD. Our recent description of ISS-related MR as a prominent underlying cause for WHF 'pathological' MR highlighted the weakness of a screening strategy based firmly on an MR severity assessment. Therefore, rather than complicate the current MM criteria by incorporating a potentially complex mechanistic assessment to identify ISS-related MR cases, we opted for a strategy based on removing the current MR severity assessment in favour of a pure morpho-mechanistic assessment of the MV.

Through our experience with RHD screening in the Echo in Africa project (EIA), the largest on-going RHD screening program in South Africa, we have developed a set of screening criteria that principally evaluate leaflet morphology, -motion and mechanism of regurgitation; the morpho-mechanistic (MM) diagnostic RHD screening criteria (

7.8. Tables

Table 7. 1 for synopsis of MM criteria; see supplementary material-
Addendum A (i) for comprehensive description of the MM criteria). For the evaluation of the MV, a novel, non-measurement-based assessment is used to recognise 'typical' distal-tip thickening of the anterior mitral valve leaflet (AMVL).[4] Thereafter, the AMVL and posterior mitral valve leaflet (PMVL) are assessed for the presence of typical rheumatic diastolic restriction as per a strict, novel screening definition. Finally, a mechanistic evaluation of MR is made, should it be present. For the aortic valve (AV), the presence of any aortic regurgitation (AR) was, in the EIA experience, a very infrequent finding outside of the context of a bicuspid aortic valve (BAV) with a much smaller mechanistic differential and complexity to consider. Consequently, normal variants and alternative aetiologies mimicking rheumatic AR appear less problematic in the evaluation of this valve. Therefore, the presence of non-BAV AR features as an essential element of our AV RHD criteria supported by typical AV leaflet restriction and rheumatic AV leaflet tip thickening.

In addition to an assessment of the MM criteria for diagnosing RHD, we aim to assess the performance of an abbreviated MM RHD rule-out test aimed at improving screening efficiency in the field. A 'screen-negative' test is defined by 1) the absence of AMVL restriction affecting the central portion (A2) of the mitral valve and 2) the absence of any aortic regurgitation (AR). The specific RHD rule-out screening criteria were selected for the MV and AV based on observations made in a recent assessment of high-and very low-risk RHD cohorts from the EIA project (see Chapter 6; The variable spectrum of anterior mitral valve leaflet restriction in rheumatic heart disease screening). In this study, a strict definition of AMVL restriction, assessed in the central portion of the AMVL, allowed for the screen inclusion of all WHF 'definite RHD' MV cases identified in EIA without inflating screened numbers from over-selecting very low-risk cases.

The lack of a confirmatory test for RHD complicates the development and validation of alternate screening approaches that aim to revise aspects of the consensus-driven WHF guideline. Currently, the only reasonable means to gauge the performance of novel RHD screening criteria would be to apply them in two cohorts of patients: one with a very low- and the other with a very high-pre-test probability (risk) for RHD. The concept of evaluating the 'false-positive' rate of RHD screening criteria using a very low-risk population were it is deemed reasonable to define all cases as RHD-negative, is well established.[5–7] However, no study has assembled a cohort of screened patients with a sufficiently high risk for RHD (given the relatively low absolute risk found in even very-high risk communities of ~ 1-5%)[6,8] to compare the false-negative rate of novel screening criteria alongside the WHF. We hypothesised that a patient with a proven diagnosis of prior acute rheumatic fever (ARF) and with current echocardiographic evidence of appreciable valvular dysfunction would represent an externally validated case with the highest possible pre-test probability for RHD so that it would be reasonable to define all cases in this cohort as RHD-positive (gold standard RHD-positive cohort). Accordingly, these cases would represent a composite reference standard with which to assess the false-negative rate of current screening criteria.[9] A composite reference standard is constructed when the results from an imperfect test [Jones/Modified Jones criteria] are combined with a predetermined rule (functional valvular deficit; significant MR or AR) to form a gold standard cohort that can be used to evaluate the index test.

The current study aims to assess the performance of the MM diagnostic screening criteria for the diagnosis of RHD in addition to assessing the performance of a screening rule-out test for RHD. These assessments will be done alongside the WHF criteria in a gold standard RHD-negative cohort where the prevalence of RHD is defined to be 0% (to estimate false-positive rate) and in a gold standard RHD-positive cohort, where the prevalence of RHD is defined to be 100% (to estimate false-negative rate).

7.3. Methods

Generation of a gold standard RHD-negative cohort

Study design, setting and participants

A retrospective analysis of echocardiographic data captured from 364 healthy secondary school children (aged 13-18 years) was performed. All scholars attended an affluent, independent (private) secondary school situated in the Cape Winelands District Municipality. An a priori hypothesis assumed that the RHD risk profile of attending scholars (i.e. risk of poverty, overcrowded households and poor access to adequate healthcare) was very low. Ethical approval for the study was obtained through the University of Stellenbosch (S17/02/030) and the governing body of the school. Written parental/guardian consent was required before study enrolment.

Screening procedure

All enrolled participants underwent an initial transthoracic echocardiogram (TTE) screening study with a handheld (HH) device (GE Medical Systems, Milwaukee, Wisconsin, USA) using a 1.7- to 3.8MHz transducer probe (G3S). All HH studies were performed according to a pre-defined study protocol (see supplementary material-Addendum A). Children with an 'abnormal' screening study (mitral regurgitation jet length ≥1.5cm, aortic regurgitation jet length ≥ 0.5 cm or any WHF morphological features of RHD) underwent a comprehensive TTE with a standard portable machine (GE Vivid I, Milwaukee, USA) with a 2- to 3.6 MHz transducer probe (GE 3S). All echocardiographic assessments were performed by the primary investigator (LDH) using a pre-defined screening protocol (see supplementary material-
Addendum **B**).

Generation of a gold standard RHD-positive cohort

Study design, setting and participants

An ARF registry database managed at Tygerberg Academic Hospital was searched for individuals with a proven Jones/Modified Jones-positive[10] diagnosis of ARF. This registry comprises of study participants sourced from three national provinces (Western Cape, Eastern Cape and Kwa-Zulu Natal). All potential study participants were contacted and offered inclusion in the study. One-hundred and nine (109) study participants (aged 7-48 years) with a documented prior history of ARF were prospectively enrolled for this study. Approximately 50% of patients with prior ARF have concurrent carditis, leading to RHD, thus making this a very high-risk population. To identify ARF cases with concomitant RHD from associated carditis, an additional criterion was added before inclusion into the gold standard RHD-positive cohort. All proven ARF cases required additionally, an MR jet ≥2cm or an AR jet ≥1cm for inclusion into the gold standard RHD-positive cohort. As required by current screening guidelines, patients with identified alternative pathologies explaining the degree of MR/AR were excluded. Written consent was required before study enrolment. Ethics approval for the study was obtained from the relevant Health Sciences committee at the University of Stellenbosch (S17/02/030) and each relevant provincial ethics board. Study enrolment took place in a private consultation room at the study participant's nearest State health facility.

Screening procedure

Each participant underwent an initial HH screening TTE, followed by a comprehensive TTE regardless of the initial HH findings. All echocardiographic assessments were performed by the primary investigator (LDH) using

the same echocardiographic machines and screening protocol, as previously described in the gold standard RHD-negative cohort screening procedure.

Echocardiogram analysis strategy and echocardiographic definitions

All paired TTE studies (HH and comprehensive) captured from both cohorts were deidentified and uploaded to an EchoPAC™and V-scan Gateway™ database for subsequent off-line analysis. All paired studies were reviewed by the lead investigator (LDH) according to a standardised protocol. All data were entered directly into a Redcap electronic data-capture system hosted at Stellenbosch University.[11]

An initial analysis was performed to determine the classification of each case (definite, borderline or normal) according to WHF-and MM criteria and was compared to the composite reference standard for each case (previous proven ARF and MR≥2cm and/or AR≥1cm). A second analysis was performed to better understand the performance of the WHF- and MM-criteria in their individual assessments of each valve (MV and AV assessed independently for ruling into gold standard RHD for that valve. In this analysis, the classification of each valve was determined as definite, borderline or normal according to the WHF-and MM- criteria and was compared to the composite reference standard for each valve (composite reference standard for rheumatic MV: prior ARF with MR≥2cm and composite reference standard for rheumatic AV: prior ARF with AR≥1cm).

All participants who had undergone previous valve repair/replacement were excluded from the gold standard RHD-positive cohort. However, the unoperated valve was included in the second analysis evaluating each valve in isolation if it fulfilled the requirements of the composite reference standard.

All analyses were done post hoc by extracting each echocardiographic feature, as measured and recorded by the lead investigator and combining features to determine which WHF/ MM screening criteria were met. Similarly, the performance of the MM ‘rule-out’ test was determined retrospectively. Measures were put in place to address concerns of recall bias. Firstly, the average time interval between the initial study capture and read in each case was >12 months. Secondly, all paired studies were deidentified and then randomised within a single cohort, thus blinding the reader to the underlying risk profile of each case.

Assessment of interobserver agreement

To assess the interobserver agreement related to the MM criteria, a random subset of 75 screening studies from each cohort was de-identified, randomised and read by a second reader (PGH) who was blinded to the initial screening results. The reader was required to classify each case as normal, borderline or definite according to the MM criteria after cases were deidentified.

Statistical analysis

Study data were collected and managed using the REDCap electronic data-capture system hosted at Stellenbosch University.[11] Statistical analyses were performed using MedCalc for Windows version 12.0 (MedCalc Software, Mariakerke, Belgium). As we used a pre-existing cohort of screened children with a very low-risk for RHD and patients with a previous history of ARF, no prespecified sample size calculation was performed. Prevalence of borderline RHD and definite RHD are reported as percentages and 95% confidence intervals (CI). Differences between the prevalence of borderline- and definite RHD were compared between both cohorts using a Fisher exact test. In all two-tailed statistical tests, P values < .05 were considered to indicate statistical significance. The sensitivity and specificity of both criteria was calculated with 95% confidence intervals (CI). Cohen's kappa statistic was used to evaluate the inter-rater agreement between the lead investigator and a second reader. The interpretation of kappa values was based on the Landis and Koch guidelines.[12] The proportion of agreement was reported as mean percentages with a 95% CI for inter-rater agreement.

7.4. Results

Gold standard RHD-negative cohort analysis

A total of 364 school children had a screening echocardiogram (Figure 1). Of these, 33 children were identified with an 'abnormal' HH screening study and underwent a second comprehensive TTE study. A BAV was identified in three children in this cohort, and these cases were excluded from further analysis. There were no cases of mitral valve prolapse (MVP). Therefore, the MM- and WHF- criteria were applied in 30 cases.

The prevalence of MM 'borderline RHD' in the gold standard RHD-negative cohort was 2.7 cases per 1000 population (95% CI,0.5-15.4). In comparison, the prevalence of WHF 'borderline RHD' was 41.8 cases/1000 (95% CI,25.5-67.8; P<0.0004). Out of the 14 cases identified with WHF 'borderline RHD'- subcategory B; isolated 'pathological' MR), 11 cases (78.5%) had an MR mechanism consistent with ISS-related MR. No explicit, identifiable mechanism of MR was found in the three remaining cases with isolated 'pathological' MR. Similarly, no morphological features of RHD (by WHF- or MM-criteria) were present in these cases. A single case of borderline AV disease was identified by both criteria. In this case, there was a central AR jet ≥1cm that met WHF criteria for 'pathological' AR. However, the valve morphology was normal with normal leaflet motion. Overall, the screening specificity of the MM- and WHF-criteria was 99.7% (95%CI, 98.4-99.9) and 95.9% (95%CI, 93.2-97.7) respectively. The application of the MM 'rule out' screening test excluded 359 cases (98.6%). Of the five remaining cases, four cases had a degree of AR that was unrelated to a BAV and one case was identified with 'distal tip' AMVL restriction without associated leaflet thickening or PMVL restriction.

Gold standard RHD-positive cohort analysis

A total of 109 participants with a previous history of prior, proven ARF had a screening echocardiogram (Figure 2). As per the study protocol, all participants underwent a comprehensive TTE study. Fifteen (15) patients with prior single valve replacement were excluded from consideration for the gold standard RHD-positive cohort.

Twenty-six (26) participants had MR<2cm and/or AR <1cm on their comprehensive TTE and were also excluded from consideration for the gold standard RHD-positive cohort. Of these 26 cases, 24 cases were diagnosed as being normal by both the WHF- and MM criteria, representing 22% (24/109) of the study participants with prior, proven ARF. Of the two remaining cases, there was discordance in diagnosis between the WHF- and MM-criteria. In one case, the MM criteria diagnosed definite disease of the MV while the WHF criteria diagnosed borderline disease of the MV. In this particular case, there was AMVL thickening with central 'distal tip' AMVL restriction and focal PMVL restriction with an MR jet length of 1cm and an incomplete continuous wave (CW) Doppler envelope thus ruling the patient out for WHF 'definite RHD'. In the other case, the MM criteria diagnosed borderline disease of the AV, while the WHF criteria diagnosed the case as being normal. This case demonstrated thickening and restriction of the AV leaflets with an AR jet length <1cm and an incomplete CW Doppler envelope.

Sixty-eight (68) of the 109 ARF participants had MR≥2cm and/or AR≥1cm on their comprehensive TTE. Assessing these 68 cases for alternative pathologies, revealed two cases with MVP and one case with a severe dilated cardiomyopathy (DCMO) with a mechanism of MR consistent with leaflet tethering. The two MVP cases displayed typical prolapse-spectrum leaflet billowing with myxomatous morphology. There were no associated morphological rheumatic changes and the AV was normal in both cases. We considered these true prolapse-spectrum cases and excluded them from further analysis.
Three of the remaining 65 cases fulfilled criteria for MVP. Two of the cases were evaluated shortly after an episode of active rheumatic carditis. In both cases, the leaflets were not typically myxomatous but displayed leaflet flail with severe MR. These cases were therefore included as rheumatic cases in the gold standard RHD-positive cohort. In the third case, also seen shortly after an episode of acute rheumatic carditis, a prominent cleft was identified in the AMVL. Although the morphology was compatible with a congenital AMVL cleft, we viewed this as a possible AMVL tear related to the recent episode of acute carditis and included the case in the gold standard RHD-positive cohort. No cases with BAV were identified in the very high-risk cohort.

Ultimately, 65 participants were assigned to the gold standard RHD-positive cohort. In the initial analysis, the MM criteria identified 61/65 cases (93.8.%,95% CI, 83.2-97.5) as definite RHD and 4/65 cases (6.2%) as borderline RHD. In comparison, the WHF criteria identified 58/65 cases (89.2%,95% CI, 79-95.6) as definite RHD, 4/65 cases (6.2%) as borderline RHD and 3/65 cases (4.6%) as normal. All three cases diagnosed as WHF normal were diagnosed with isolated AV disease according to the MM criteria. Two of the cases had restriction and thickening of the AV with an AR jet length of ≥1cm but with an incomplete CW doppler envelope (i.e. not pandiastolic). The remaining case had no morphological abnormalities of the AV with an AR jet length of ≥1cm but with an incomplete CW Doppler envelope. Overall, there was complete agreement for the diagnosis of RHD (i.e. borderline or definite disease) between the WHF- and MM-criteria criteria in all but three (4.6%) of the 65 cases within the gold standard RHD-positive cohort.

A second analysis was performed, aimed at assessing the performance of the MM- and WHF-criteria in each mitral- and aortic valve separately. Here, a composite reference standard was created for the individual valves

rather than for the overall cases using the same methodology as described previously. In the individual MV and AV assessments, 61 mitral valves had MR ≥2cm, and 33 aortic valves had AR≥1cm (with an overlap of 20 cases with both MR≥2cm and AR ≥1cm) constituting a total of 94 gold standard RHD-positive valves against which we tested the performance of the MM- and WHF -criteria.

In the isolated MV assessment, the MM criteria classified 54/61 (88.5%,95% CI,77.8-95.3) as definite, 4/61 (6.6%) as borderline, and 3/61(4.9%) as normal (Table 7. 2). In comparison, the breakdown of the WHF criteria for definite, borderline and normal mitral valves was 51/61 (83.6%,95% CI,71.9-91.9), 8/61 (13.1%) and 2/61 (3.3%) respectively. In this isolated MV evaluation, 3/61 valves were classified as normal, two valves by both the MM and WHF criteria and the other by the MM criteria only. There were no morphological rheumatic changes affecting any of the three valves making these all isolated MR cases. A mechanistic evaluation of the cause of regurgitation identified an ISS as the mechanism of MR in two cases and an unclear mechanism in the third case.

In the isolated AV evaluation, the criteria performed as follows: MM ‘definite RHD’ in 30/33 cases (90.9%,95% CI,75.7-98), MM ‘borderline RHD ’in 3/33 cases (9%). The WHF classified WHF ‘definite RHD’ in 27/33 cases (81.8%,95% CI,64.5-93), WHF ‘borderline RHD’ in 2/33 cases (6%) and WHF ‘normal’ in 4/33 cases (12.1%; Table 7. 3). The four normal WHF cases were all cases with AV morphological abnormalities and with an AR jet length ≥1cm but without the required associated Doppler criteria. In the very high-risk cohort, the application of the MM ‘rule out’ screening test excluded 30 of the 109 cases (27.5%). None of the 65 cases within the gold standard RHD-positive cohort were excluded.

Assessment of interobserver agreement

The agreement between readers for the diagnosis of MM definite disease was almost perfect substantial (kappa=0.83; 0.73-0.92) with a proportion of agreement of 92%. There were insufficient MM borderline cases to accurate determine the degree of agreement between readers. There was almost perfect agreement between readers for the diagnosis of MM normal (kappa=0.95; 0.91-1) with a proportion of agreement of 98%.

7.5. Discussion

In the present study, a set of screening criteria based primarily on a morpho-mechanistic (MM) evaluation is tested alongside the current WHF criteria for the echocardiographic detection of RHD. In a novel approach, this study established a mechanism by which the screening performance (i.e. specificity and sensitivity) of both sets of criteria could be evaluated. Based on our results, the MM criteria significantly reduced the false-positive rate of a borderline diagnosis in a gold standard RHD-negative cohort (specificity 99.7% vs 95.8%), while maintaining a similar sensitivity (92.4% vs 89.2%) in a gold standard RHD-positive cohort. Furthermore, the performance of an

abbreviated MM 'rule-out' test was excellent and excluded the majority of gold standard RHD-negative cases (98%) while identifying all patients within the gold standard RHD-positive cohort. This holds promise for the development of a two-step screening algorithm that could simplify the implementation of the MM criteria in the field. Further prospective study is required to investigate the feasibility of this screening algorithm and importantly, whether the MM criteria identify children with the highest risk for an unfavourable outcome.

A principal concern that has been raised regarding the WHF criteria relate to their identification of a large, diverse diagnostic group of children with potential disease (borderline RHD). It is now well accepted that a significant proportion of children within this group (particularly those with isolated WHF 'pathological' MR) are misclassified with RHD and represent false positives.[5,6] Current research in RHD screening has focussed on refining aspects of the current WHF criteria and seeking accurate echocardiographic screening approaches that could that make a diagnosis of true RHD more likely.

Recently, Nunes et al. proposed a simplified score for latent RHD diagnosis, based on components of the World Heart Federation criteria. Using logistical regression modelling, a large derivation and validation cohort were analysed to identify the WHF components that demonstrated the highest predictive value for WHF 'definite RHD'. Of the various WHF criteria, it appeared that AMVL thickening, excessive leaflet motion, an MR jet >2cm, AV thickening and any degree of aortic regurgitation (AR) were the most predictive for WHF 'definite RHD'. However, a major limitation in the approach taken in this work has been identified as incorporation bias.[13] In this bias, all selected variables representing the index test (individual components of the WHF criteria), comprised part of the gold standard test (final WHF criteria diagnosis). This is an important methodological limitation that is frequently encountered in RHD screening literature due to the lack of a confirmatory test for true RHD.[14] In the current study, we have established a novel mechanism to test the screening sensitivity and specificity of the WHF criteria alongside the MM criteria while addressing the potential impact of incorporation bias. This was facilitated by the establishment of a composite reference standard[9] (gold standard RHD-positive cohort) consisting of patients with a proven, prior history of ARF and MR≥2cm or AR ≥1cm on their current echocardiogram.

In the current study, and for the first time, we propose a set of novel morphological criteria for the echocardiographic diagnosis of RHD. We envisaged that the morphological assessment would solely rely on the identification of 'typical' rheumatic thickening and restriction of the mitral- and aortic valve as keystones for the echocardiographic identification of RHD and from which, a morpho-mechanistic evaluation, rather than a regurgitation severity score, would identify a rheumatic, from a non-rheumatic aetiology of dysfunction.

There are notable differences in the application of the morphological criteria as used in the MM criteria when compared to the current WHF criteria. Firstly, the MM criteria adopt a non-measurement AMVL thickness assessment instead of a measurement-based evaluation relying on a strict cut-off value. This has been demonstrated to be poorly reproducible.[15] Furthermore, it is a complex and time-consuming assessment and not conducive to screening in the field. Secondly, the MM criteria provide strict, echocardiographic definitions for

leaflet restriction of the MV and AV. Here, the most prominent is a novel definition that has been developed to recognise subtle RHD-related 'distal tip' AMVL restriction (see Chapter 6; The variable spectrum of anterior mitral valve leaflet restriction in rheumatic heart disease screening). Thirdly, the criterion of 'excessive leaflet tip motion' was not included in the MM assessment. It is our experience that this current criterion creates confusion, as it describes both prolapse-spectrum pathology as well as 'pseudoprolapse' of the AMVL.

Mitral valve prolapse (outside of the typical non-rheumatic myxomatous prolapse-spectrum disease) was not identified in any of the 5225 asymptomatic children enrolled in the EIA project. It was, however, present in three cases associated with leaflet flail and severe MR in the gold standard RHD-positive cohort described in this study. Furthermore, the morphology of the 14 valves that were repaired/replaced is not known and conceivably more RHD-related leaflet flail could have made it into this population. It seems likely that cases with leaflet flail would present early after ARF with severe valvular regurgitation, as one would expect the risk of chordal rupture to be highest during the acute phase of valvulitis from ARF. This explains the high prevalence of this lesion described in surgical cohorts[16] and was in fact the pattern identified in our cohort as well where all cases with flail were evaluated shortly after an episode of clinical ARF-related carditis. In comparison to this, pseudoprolapse of the AMVL was a common mechanism of RHD-related MR in the gold standard RHD-positive cohort in the current study. This also holds true in the more-typical high-risk screening populations where pseudoprolapse remains the most common mechanism of RHD-related 'pathological' MR when using the WHF criteria (see Chapter 4: Echocardiographic assessment of subclinical rheumatic heart disease: The Echo in Africa project).

Pseudoprolapse of the AMVL is an important mechanism of RHD-related MR which does not represent true prolapse but rather describes cases where the tip of the AMVL appears to move excessively relative to the PMVL tip, but importantly, remains well above the MV annulus.[16,17] The underlying mechanism here is typically not true excessive AMVL tip prolapse but rather represents a degree of PMVL restriction. Lastly, the WHF criteria of 'chordal thickening', 'coaptation defect of the AV' and 'leaflet prolapse of the AV' were not considered for inclusion. The prevalence of all these entities were extremely low in our large-scale screening project (EIA), and if present, were associated with additional features of MV/AV disease that would clinch a WHF 'definite RHD' diagnosis (see Chapter 6; The variable spectrum of anterior mitral valve leaflet restriction in rheumatic heart disease screening).

In the gold standard RHD-negative cohort, the MM criteria performed well by only identifying a single case with MM 'borderline RHD' of the AV. This case in all likelihood represented a true false-positive study as the AR was very mild and the underlying AV morphology and mobility were normal. Consequently, the specificity of the MM criteria was excellent (99.7%). In comparison, the WHF criteria identified 14 cases with isolated 'pathological' MR in addition to the case with isolated AV regurgitation that was identified by the MM criteria. Interestingly, an underlying mechanism of ISS-related MR was identifiable in 11 of the 14 cases or 78.5% of the total WHF

borderline group. Crucially, no morphological features of RHD (by WHF- or MM criteria) were detected in these cases nor the remaining three cases with isolated WHF 'pathological' MR. The difference in prevalence of MM- and WHF-detected cases within the gold standard RHD-negative cohort was statistically significant ($p<0.0004$), reflecting an almost twentyfold increase in 'screen-positive' cases. This amounts to roughly an additional 40 cases/1000 screened that would require detailed scanning by an expert and additional resource intensive follow-up. The size of the borderline group is an important driver for the need for resource intensive follow up and if not kept in check, has the potential to cripple a large-scale screening program driven by non-expert field workers.

The high sensitivity of the WHF criteria for definite disease is well accepted, however before now, this has never been directly assessed.[1,5] In this study, the WHF criteria performed well in this regard, and identified a high proportion of cases with WHF 'definite RHD' (89.2%) within the gold standard RHD-positive cohort. The MM criteria performed equally well and identified an additional three cases with MM 'definite RHD'(92.4%).

Importantly, the MM criteria (borderline and definite categories) did not miss any gold standard RHD-positive cases as compared to the WHF criteria where 3 cases were classified normal. This is an important metric in RHD screening as false-negative results (reducing sensitivity) have even more serious implications for the screened patient than false-positive results (low specificity).[5] This was scrutinised in even more detail in the second analysis undertaken in this study where the two sets of criteria were assessed for performance in the mitral and aortic valves separately. In the isolated MV assessment (Table 7. 2), three cases with MR ≥2cm were identified as normal by the MM criteria compared to two cases that were identified by the WHF criteria as normal. In all three cases identified by the MM criteria, the underlying MV leaflet morphology and motion was normal with a mechanism of MR that was attributable to an ISS identified in two of the cases whilst a mechanism of MR was unclear in the third case. After careful scrutiny, these 3 cases were considered to be false-negative cases as they reflect normal, non-rheumatic mitral valves. This makes the baseline prevalence of ISS-related MR of 2 cases/61 (3.3%) in the gold standard RHD-positive cohort roughly approximated the prevalence identified in the gold standard RHD-negative cohort of 14 cases/364 (3.8%).

In the isolated AV assessment (Table 7. 3), four cases with true AV disease were diagnosed as WHF 'normal'. In all four cases, the MM criteria diagnosed MM 'definite RHD' of the AV. There was leaflet thickening and restriction noted in all cases, yet the AR regurgitant jet was not pandiastolic and had an incomplete CW Doppler envelope. Therefore, all four cases missed a WHF 'definite RHD' diagnosis due to a technicality, rather than a true absence of disease, driven primarily by the complexity of the additional Doppler requirements of the WHF criteria.

The abbreviated MM RHD rule-out screening test performed remarkably well despite its simplicity. Importantly, there were no false-negative cases within the gold standard RHD-positive cohort and the false-positive rate within the gold standard RHD-negative cohort was extremely low (5/364;1.4%). This translates into a low requirement for re-screening and follow-up. We postulate that this screening test could represent an ideal initial step in a two-

step screening algorithm for the echocardiographic detection of RHD. The screening methodology for identifying central 'distal tip' AMVL restriction and any AR is relatively simple and can be performed by an upskilled healthcare worker/non-expert screener. Crucially, the rule-out test can be implemented with a HH screening device where all components could be reliably assessed in a single PSLAX view. This would allow for task shifting for RHD screening where a non-expert could efficiently screen high-risk children in RHD endemic regions.

Limitations

The borderline category assessed with both sets of criteria in this study derive primarily from the gold standard RHD-negative cohort and consist primarily of cases with isolated WHF 'pathological' MR. It is conceivable that a borderline group derived from a high prevalence cohort may look different in terms of having more minor morphological changes rather than a primarily functional pathology and as such, these results may not be representative. A separate borderline cohort from a high-risk screening study was not included in the cases assessed in the current study. The problem with including a borderline cohort from a high-risk screening study is that there is no externally validated means of moderating the results of such 'screen- positive' cases. However, the borderline group from a number of high-risk screening studies including our own (EIA project), had a similar, predominantly isolated 'pathological' MR, makeup as was seen in the gold standard RHD-negative cohort here. Furthermore, we anticipate the MM criteria to be particularly well suited to identifying borderline cases with rheumatic morphological changes being primarily focussed on a morphological assessment.

7.6. Conclusion

This study evaluates the performance of a novel set of echocardiographic MM screening criteria alongside the current WHF criteria for the detection of RHD. The MM criteria significantly reduced the false-positive rate of a borderline diagnosis in a gold standard RHD-negative cohort whilst maintaining a similar screening sensitivity within a gold standard RHD-positive cohort. Finally, the performance of an abbreviated RHD rule-out test was notable for its ability to exclude 98% of cases from the gold standard RHD-negative cohort while including all cases within the gold standard RHD-positive cohort. This holds promise for a two-step screening algorithm that would enable non-expert screeners to effectively screen for latent RHD. Further prospective study is required to investigate the feasibility of this approach and importantly whether the MM criteria identify those at highest risk for an unfavourable outcome.

7.7. References

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7.8. Tables

Table 7. 1. Morpho-mechanistic criteria for echocardiographic detection of rheumatic heart disease

Diagnostic scoring for MM 'definite RHD'-, 'borderline RHD'- and normal-cases

Scores ≥4 define a diagnosis of definite RHD, scores of 2 and 3 define a diagnosis of borderline RHD, scores of 0 and 1 identify a non-rheumatic normal valve. Borderline MM disease of both the mitral- and aortic-valve constitute a diagnosis of MM 'definite RHD'.

Mitral valve assessment	**Score**
Morphological abnormalities	
1. AMVL thickening	1
2. Restriction of leaflet motion	
• Central 'distal tip' AMVL restriction	1 point if no AMVL thickening, 3 points if AMVL is thickened
• PMVL restriction	1
• Central 'gradual bowing' AMVL restriction	1
• Mitral stenosis	4

Mechanistic assessment (if MR present)	
• Pseudoprolapse mechanism of MR	1
Aortic valve assessment	
Morphological abnormalities	
1. Restricted leaflet motion	1
2. Leaflet thickening	1
Functional assessment	
1. AR ≥1cm	3

AMVL, anterior mitral valve leaflet; PMVL, posterior mitral valve leaflet; MV, mitral valve; MR, mitral regurgitation; AR, aortic regurgitation

Table 7. 2. Assessment of the WHF- and MM criteria against a composite reference of all gold standard RHD-positive mitral valves.

Mitral valve*	MM criteria	WHF criteria
Definite(n)	54	51
Borderline(n)	4	8
Normal(n)	3	2
Total(n)	61	61

*Mitral valve cases in ARF cohort with MR jet ≥2cm

MM, morpho-mechanistic; WHF, World Heart Federation; ARF, acute rheumatic fever

Table 7. 3. Assessment of the WHF- and MM criteria against a composite reference of all gold standard RHD-positive aortic valves.

Aortic valve*	MM criteria	WHF criteria
Definite(n)	30	27
Borderline(n)	3	2
Normal(n)	0	4
Total(n)	33	33

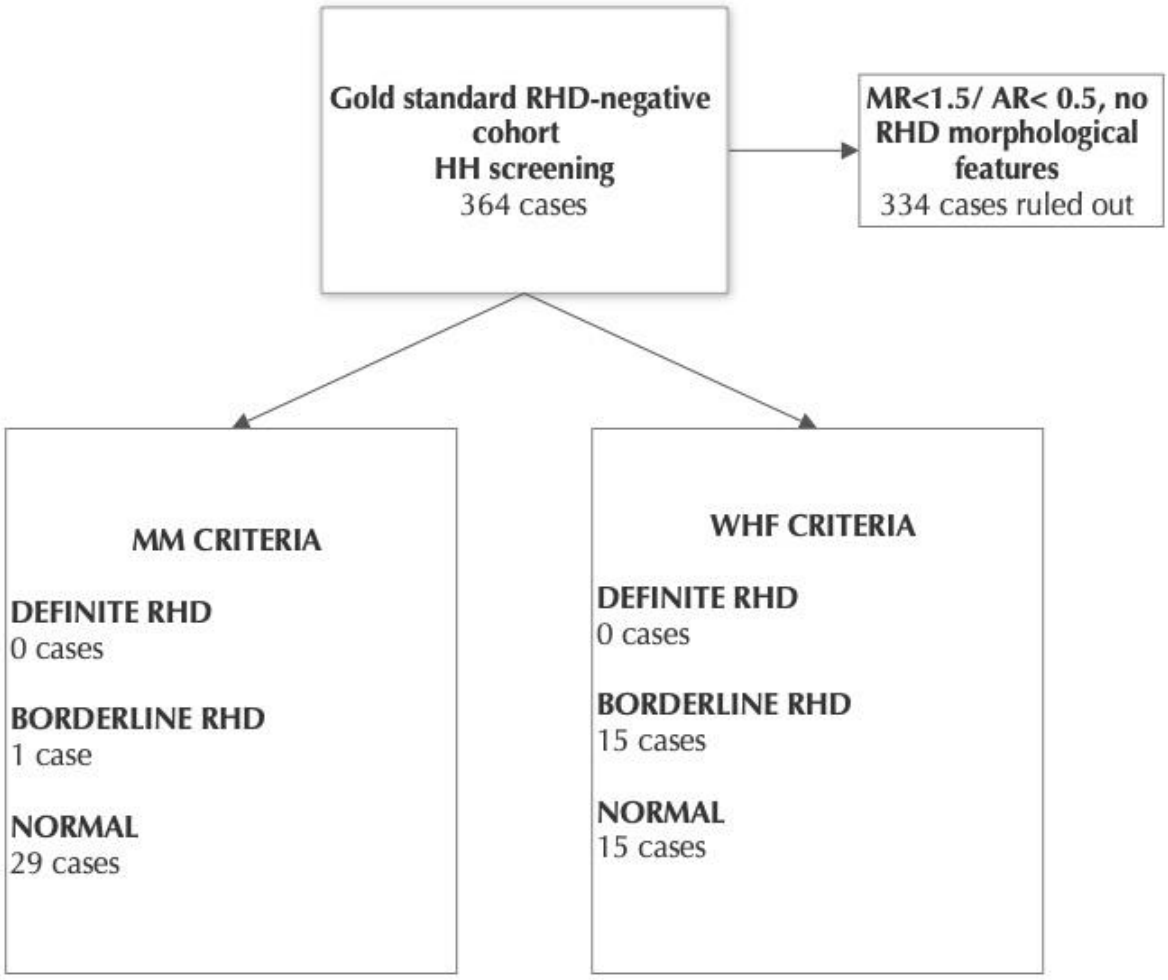
*Aortic valve cases in ARF cohort with AR ≥1cm

MM, morpho-mechanistic; WHF, World Heart Federation; ARF, acute rheumatic fever

Figures

Figure 1

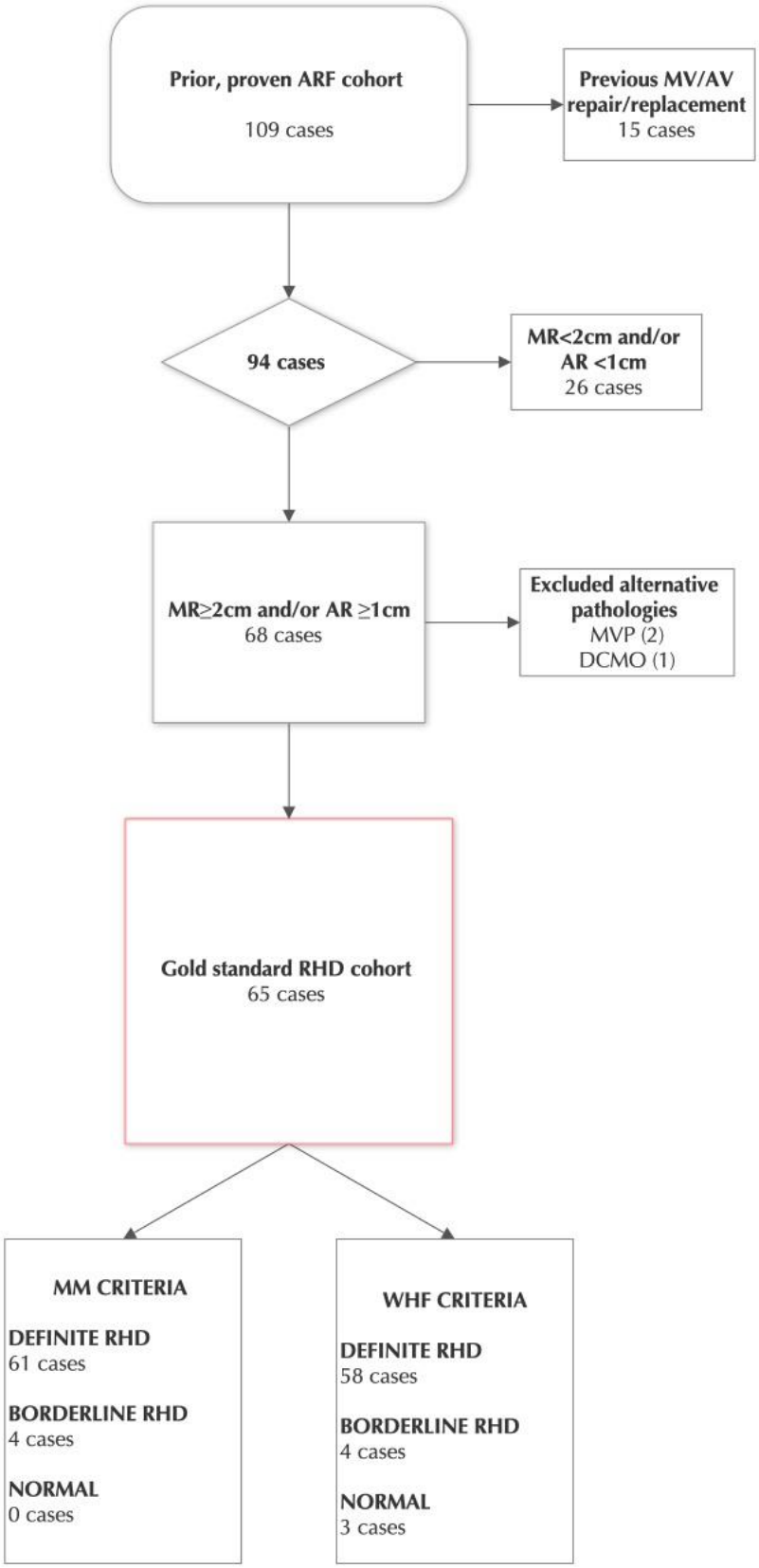
Generation of a gold standard RHD-negative cohort



MM, morpho-mechanistic; WHF, World Heart Federation; HH, handheld; MR, mitral regurgitation; AR, aortic regurgitation; BAV, bicuspid aortic valve; RHD, rheumatic heart disease; TTE, transthoracic echocardiogram

Figure 2

Generation of a gold standard RHD-positive cohort from a very high-risk cohort



TTE, transthoracic echocardiogram; MV, mitral valve; AV, aortic valve; MR, mitral regurgitation; AR, aortic regurgitation; MVP, mitral valve prolapse; DCMO, dilated cardiomyopathy; RHD, rheumatic heart disease

7.9. Supplementary material

Addendum A (i)

Morpho-mechanistic criteria for echocardiographic detection of rheumatic heart disease

The MM criteria are a quantitative diagnostic scoring system for the echocardiographic diagnosis of RHD. The mitral-(MV) and aortic valve (AV) are evaluated separately and are scored based on the presence/absence of specific morpho-mechanistic features of RHD.

Morphological assessment of the MV

1. **AMVL thickness assessment**

For the RHD screening evaluation of the MV, we constructed a set of criteria leading from an AMVL thickness assessment. Here, the central portion of the AMVL (A2) in the parasternal long-axis (PSLAX) view is assessed for the presence of 'typical marked thickening' of the distal tip. The leaflet assessment is performed in PSLAX view. The cine image is stopped and scrolled to find the position of maximal diastolic leaflet extension. It is important to assess the central (A2) portion of the leaflet. This is typically identified by sweeping across the leaflet in the PSLAX image and settling in a central position devoid of the more lateral and medial strut chord implantation sites. This makes the assessment of leaflet thickness separate from chordal interference easier. The thickness assessment done in this position is non-measurement-based and relies on an assessment of leaflet tip thickness in relation to the thickness at the leaflet base. In normal valves, the leaflet tip and base differ very little in thickness and pragmatically, when the tip of the central AMVL is visually assessed to be more than twice the basal leaflet thickness in such an optimised frozen image, tip thickening is identified. A thickened AMVL scores 1 point in the MM assessment. Note: the assessment of AMVL tip thickening is the first step in the application of the criteria as it influences subsequent scoring.

2. **Restriction of MV leaflet motion**

AMVL restriction is defined when the tip of the AMVL points away from the left interventricular septum towards the posterior left ventricular (LV) wall during peak diastolic leaflet extension in the PSLAX view. The leaflet configuration in each case should be closely scrutinised to distinguish so-called 'distal tip' AMVL restriction from 'gradual bowing' AMVL restriction (see Chapter 5, The variable spectrum of anterior mitral valve leaflet restriction in rheumatic heart disease screening). 'Distal tip' AMVL restriction is scored 3 points in the presence of AMVL tip thickening but only scores 1 point in a non-thickened leaflet. Similarly, 'gradual bowing' restriction affecting the central AMVL (A2) scores only 1 point. Importantly, no points are allocated for restriction that is seen to only affect the very medial and lateral aspects of the AMVL.

An assessment of posterior mitral valve leaflet (PMVL) is performed next. PMVL restriction is defined when the tip of the leaflet points towards the interventricular septum during maximal diastolic PMVL extension in the PSLAX view. Typically, when only the leaflet tip is restricted, bowing of the leaflet

ensues. However, the degree of leaflet mobility can be variable and when severely restricted, the PMVL becomes immobile. PMVL restriction (either bowing or immobile) scores 1 point.

3. **Mitral stenosis**

Mitral stenosis (MS) represents a pathognomonic lesion of RHD. Alternative causes are rare and include mitral annular calcification (MAC) in elderly patients and rare congenital causes such as 'parachute' mitral valve. MS in children, where extensive calcification renders the valve immobile is very rare. Leaflet thickening and bowing (representing restriction) is essentially always present. Therefore, the inclusion of gradients or specific valve areas were not included here, as these valves rule in by the above-noted morphological criteria of leaflet thickening and leaflet restriction.

Mechanistic assessment of mitral regurgitation

A mechanistic evaluation based on a Carpentier-style assessment of the MV is performed on all cases identified with MR. (see Chapter 2, Inter-scallop separations of the posterior mitral valve leaflet: a solution to the 'borderline RHD' conundrum?). Briefly, the underlying morphology of the MV should be assessed for features of mitral valve prolapse / prolapse spectrum (excessive leaflet motion), congenital AMVL clefts and inter-scallop separations (ISS) of the PMVL (normal leaflet motion) and for features of pseudoprolapse of the AMVL (restricted leaflet motion). If the mechanism of MR is consistent with pseudoprolapse, then the MV scores 1 point. Note: an MR severity assessment does not form part of the MM criteria.

Morphological assessment of the aortic valve

1. Aortic valve restriction

The normal aortic valve (AV) leaflets open parallel to the aortic wall at full excursion. AV restriction is defined when the AV leaflet tip points away from the aortic walls and toward the aortic lumen at maximal systolic extension. The assessment is performed in the PSLAX view, incorporating a so-called parasternal sweep to visualise the morphology of the right-, left- and non-coronary cusps. (screening technique described in Chapter 2, Inter-scallop separations of the posterior mitral valve leaflet: a solution to the 'borderline RHD' conundrum?). A parasternal short-axis view should always follow to exclude a bicuspid valve (BAV) or associated spectrum. If a BAV is confirmed, then the AV should be excluded from further assessment, whilst the mitral valve be subjective to a formal analysis as described above. AV restriction scores 1 point.

2. Aortic valve thickening

AV thickness is assessed for the typical AV leaflet tip thickening seen in RHD. Due to the small size of the AV leaflet and poor measurement reproducibility, the assessment for AV thickening is subjective and involves in comparing tip thickness to that of the leaflet base which is typical spared. AV thickening scores 1 point.

Assessment of aortic regurgitation

For the purposes of this article, we have included the same length measurement requirement as the WHF criteria (≥1cm) for identification of 'screen-significant' AR. This was done to limit introducing a differential incorporation bias with regards to the WHF criteria in this study. The WHF criteria require an AR jet length of 1cm which is also used in this study to select patients for the gold standard RHD cohort (incorporation bias). The inclusion of the a ≥1cm requirement for the MM criteria avoids skewing of results when comparing the two sets of criteria. Outside of the context of this study, we view any AR as being significant as it is a rare finding in screening situation (see Chapter, The Echo in Africa project). AR≥1cm scores 3 points.

Summary and conclusions

Screening echocardiography, guided by the current World Heart Federation (WHF) criteria, is recognised as the diagnostic investigation of choice for the detection of 'latent' RHD in asymptomatic, high-risk children. The early detection and initiation of secondary prophylaxis in affected individuals remain attractive primary health care interventions, particularly in endemic regions with no or limited access to specialist cardiac services. However, the WHF criteria are limited by four significant shortcomings that have negatively impacted on its screening performance and have curtailed the implementation of large-scale, population-based screening. Firstly, the current guideline is complex which limits its application by non-experts in-the-field. Secondly, the criteria incorporate a Doppler-based regurgitation severity assessment that is non-specific in its identification of the underlying aetiology of dysfunction. Thirdly, the Doppler requirement of the WHF criteria make it impossible to implement using the currently available handheld devices. These devices offer many advantages over 'standalone' machines as they cheaper, more portable and battery-powered and represent an opportunity to feasibly implement affordable, large-scale screening in remoted RHD endemic regions. Finally, and most importantly, is the criteria's identification of a large borderline disease category of which a significant proportion is likely to represent false-positive cases.

To ensure that further progress is made in this field, two principal research priorities required further study. The first was to determine whether the current WHF screening criteria can be sufficiently simplified and revised to enable its efficient use by non-experts in-the-field with handheld devices. Second, was the need to address the WHF's suboptimal screening specificity by evaluating novel, alternative echocardiographic features that may better predict RHD.

To address the issues, four main research objectives were identified for this thesis. The first research objective was to analyse data from the first five years of the Echo in Africa (EIA) project, a large-scale echocardiographic screening program in the Western Cape. This collaborative program between SUNHEART (a non-profit organisation established by the Division of Cardiology at Tygerberg Academic Hospital [TBH]) together with the British Society of Echocardiography (BSE) represents the largest and only ongoing RHD screening program in South Africa. During the first five-years of EIA, a total of 5225 schoolchildren were screened for RHD, representing the largest studied cohort in South Africa. Forty-nine of the children screened were identified with WHF 'definite RHD', reflecting a definite disease prevalence of 9.1/1000. This is almost double the prevalence previously described in this region and highlights the need for developing a practical and accurate screening tool that could spearhead a RHD disease control program for high-risk schoolchildren in the Western Cape.

The second research objective was to critically appraise the WHF criteria and identify key aspects that require revision that would simplify a screening algorithm for the detection of RHD. Although initially stated as a separate entity, this research objective was intimately linked to the third objective of this thesis which was to investigate alternate morphological and mechanistic features that could better define the presence or absence of true RHD. As such, the pertinent findings and conclusions that can be drawn from both objectives are discussed here.

Based on our EIA experience, we identified that the WHF's incorporation of a non-specific Doppler-based mitral regurgitation (MR) severity score was a critical determinant in the classification of cases as WHF 'borderline RHD' (see Chapter 4). To determine the underlying aetiology of MR in children with WHF 'pathological' MR, we applied a mechanistic evaluation to the MV with the view to identify whether a known rheumatic mechanism of MR was present.

This resulted in the important discovery of inter-scallop separation(ISS)-related MR as an important cause of 'pathological' MR in the borderline group. (see Chapter 2 and 3). Based on subsequent screening in very low-and high-risk populations, we confirmed this entity as being a common, normal variant of the PMVL and a frequent underlying mechanism of clinically insignificant, yet WHF 'pathological' MR amongst our screened populations, regardless of underlying RHD risk (see Chapter 3). Furthermore, our description of 'pseudoprolapse' of the anterior mitral valve leaflet (AMVL) as the principal mechanism of MR amongst our WHF 'definite RHD' cohort (see Chapter 3) further supported the inclusion of a mechanistic evaluation of MR in a screening algorithm, rather than a non-specific Doppler-based assessment. These findings reflect an essential paradigm shift in the field and a potential opportunity to solve the current 'borderline disease conundrum'.

Based on our EIA experience, we could identify specific aspects of the current WHF morphological assessment that could be simplified. The first relates to the current WHF AMVL thickness assessment. Mitral valve (MV) leaflet thickening is a prominent feature of RHD and a crucial morphological criterion to include in a screening evaluation. However, the current measurement-

based evaluation is in our experience, a time consuming and a poorly reproducible assessment in-the-field. This we formally demonstrated in a rigorous reliability study that controlled for systematic bias (see Chapter 5). This allowed for the introduction of a non-measurement-based assessment that we found to be particularly useful in identifying 'typical' distal-tip thickening of the AMVL when using HH devices in-the-field. Here, the assessment evaluates the central portion (A2) of the AMVL and compares the thickness ratio between the basal portion of the AMVL and the distal leaflet tip.

The second important observation was that several WHF criteria were either redundant or non-contributory to the final diagnosis of definite disease. These included features such as chordal thickening, coaptation defect of the aortic valve (AV) and AV prolapse. In addition, the criterion of 'excessive leaflet tip motion' was highlighted as being ambiguous and ill-defined, risking the misclassification of cases with mitral valve prolapse and associated spectrum. Furthermore, in our EIA experience, the presence of any AR was a very infrequent finding outside of the context of a bicuspid aortic valve (BAV) with a much smaller mechanistic differential and complexity to consider. Therefore, the presence of non-BAV AR would feature as an essential element of our AV RHD criteria (and 'rule-out' test), supported by typical AV leaflet restriction and rheumatic AV leaflet tip thickening.

Finally, we identified that the lack of a specific screening definition of AMVL restriction is an important aspect that requires attention and represents an ideal opportunity to simplify the current screening criteria. Based on observations made in patients with mitral stenosis (representing the most specific lesion of RHD), we developed a novel screening definition of AMVL restriction. AMVL restriction was defined as the tip of the leaflet pointing away from the interventricular septum towards the posterior left ventricular (LV) wall during peak diastole in the parasternal long-axis (PSLAX) view. By describing the variable pattern of AMVL restriction in both our high- and very-low risk populations, 'distal tip' AMVL restriction was identified as a keystone morphological feature from which we could construct a novel set of screening criteria (see Chapter 6). The significance of central 'distal-tip' AMVL restriction became apparent when comparing the prevalence and location of AMVL restriction in a very low-, high- and very-high RHD prevalence cohort. Here, it proved to be a robust indicator of rheumatic involvement of the MV with the potential to form part of relatively simple RHD 'rule-out' test that could be taught to non-expert screeners.

The lessons learnt whilst addressing the second and third research objectives of this thesis identified the principal elements that should be included as part of a novel, simplified and improved set of screening criteria for the echocardiographic diagnosis of RHD. The final objective of this thesis was to determine whether this new set of morpho-mechanistic (MM) criteria could be validated. The absence of a confirmatory test for RHD represented a unique challenge in this final research objective. While the concept of evaluating the specificity of RHD screening criteria using a very-low-risk population is well established, no study has assembled a cohort of screened patients with a sufficiently high risk for RHD to evaluate the sensitivity. To address this, we constructed a composite reference standard (gold standard RHD-positive cohort) of patients with a prior, proven history of acute rheumatic fever (ARF) with current echocardiographic evidence of valvular dysfunction. Consequently, to estimate

both the specificity and sensitivity of the MM criteria and the performance of a 'rule-out' test, there were applied alongside the WHF criteria in a very-low risk population (gold standard RHD-negative cohort to determine false-positive rate/specificity) and a gold standard RHD -positive cohort to determine false-negative rate/sensitivity).

The MM criteria significantly reduced the false-positive rate of a borderline diagnosis in the gold standard RHD-negative cohort (specificity 99.7% vs 95.8%), while maintaining a screening sensitivity equal to the WHF criteria (92.4% vs 89.2%) in a gold standard RHD-positive cohort. Furthermore, the performance of the 'rule-out' test was notable for its ability to exclude 98% of cases from the gold standard RHD-negative cohort while including all cases within the gold standard RHD-positive cohort. This holds promise for a two-step screening algorithm that would enable non-expert screeners to effectively screen for latent RHD. Further research is required to determine the feasibility and reproducibility of this screening approach amongst non-expert screeners in RHD endemic regions.

There are important aspects of this thesis that require further prospective study. Firstly, our findings relate to a single centre's experience gained during a large-scale screening program (EIA) in the Western Cape, South Africa. The reproducibility of the MM criteria must be tested by experienced independent groups who screen high-risk populations in other regions of the world. We envisage that further collaborative work with these groups (particularly those with access to longitudinal data of children with WHF 'screen-positive' disease) would represent an invaluable research opportunity to determine the significance of our contribution. It is, of course, crucial that prospective outcome data are obtained to evaluate whether the MM criteria identify those at highest risk for an unfavourable outcome.

In conclusion, the findings of this thesis have addressed key research needs and gaps in our current understanding of 'screen-detected' latent RHD. It is anticipated that this will set the scene for the further comprehensive study of children with latent RHD with the potential to affect and influence policy on the implementation of large-scale, population-based screening in RHD endemic regions.