The Role of Mitral Valve Prolapse in Patients with Unexplained Cardiac Arrest

Wael Abdulrahman A. Alqarawi

Thesis submitted to the University of Ottawa
in fulfillment of the requirements for the M.Sc. degree in Epidemiology

School of Epidemiology and Public Health

Faculty of Medicine

University of Ottawa

ABSTRACT

Mitral valve prolapse (MVP) is thought to be one of the causes of unexplained cardiac arrest (UCA). However, previous studies are limited by the lack of a standardized evaluation of UCA and the absence of a control group to identify predictors of cardiac arrest. We performed a systematic review of studies that examined the yield UCA evaluation. We then reported the prevalence and characteristics of MVP patients from a multi-centre registry of patients with UCA. Lastly, we completed a protocol of a matched case-control study aiming at comparing echocardiographic features of MVP patients with and without cardiac arrest. As a result of these studies, we proposed a standardized algorithm for UCA evaluation and a definition for idiopathic ventricular fibrillation. Also, we reported the prevalence of MVP in patients with UCA and described few features that could potentially help distinguish patients with MVP at risk for cardiac arrest.

EXECUTIVE SUMMARY

Mitral valve prolapse (MVP) has been reported to be a potential cause of cardiac arrest, even in the absence of left ventricular dysfunction or mitral regurgitation (MR).[1, 2] However, these reports are hindered by the incomplete evaluation to role out concealed causes of cardiac arrest and/or referral bias. Moreover, while the characteristics of patients with MVP as the only cause of cardiac arrest have been described before, no studies have identified predictors of cardiac arrest that distinguish these patients from MVP patients who have a benign prognosis. In this thesis, we first performed a systematic review and meta-analysis of studies that examined the yield of comprehensive cardiac evaluation of patients with unexplained cardiac arrest (UCA), defined as cardiac arrest with no cause identified on initial evaluation. We analyzed the yield of tests incorporated in these studies and proposed criteria to assess the completeness of the evaluation which patients with UCA receive before the cause of their cardiac arrest is set to be unknown [i.e. before diagnosing idiopathic ventricular fibrillation (IVF)]. We used these criteria for our subsequent study and suggested using them in all studies examining the association between MVP and IVF, as it has been shown that this association depends on the rigors to which alternative causes of cardiac arrest has been performed.[1, 3, 4] We then described the prevalence and characteristics of patients with MVP who sustained UCA from the Cardiac Arrest Survivors with Preserved Ejection Fraction Registry (CASPER), which is a national registry of patients who suffered UCA.[5] We described 2 distinct phenotypes of patients with MVP and cardiac arrest: one of which where MVP was likely the cause of cardiac arrest (i.e. arrhythmic mitral valve prolapse, AMVP) and the other where MVP was likely an innocent bystander. We found that definite MVP is indeed associated with IVF. Lastly, we completed a protocol for a matched

case-control study that aims at identifying echocardiographic predictors of cardiac arrest in MVP patients by comparing pre-specified echocardiographic features of patients with and without cardiac arrest. The goal of this is to identify patients with MVP who are at high risk for developing cardiac arrest in the general population to institute primary preventive therapies such as implantable cardioverter-defibrillator (ICD).

References

1. Sriram CS, Syed FF, Ferguson ME, et al. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. *J Am Coll Cardiol.* 2013;62:222-230.

2. Nalliah CJ, Mahajan R, Elliott AD, et al. Mitral valve prolapse and sudden cardiac death: a systematic review and meta-analysis. *Heart.* 2019;105:144-151.

3. Basso C, Perazzolo Marra M, Rizzo S, et al. Arrhythmic Mitral Valve Prolapse and Sudden Cardiac Death. *Circulation.* 2015;132:556-566.

4. Narayanan K, Uy-Evanado A, Teodorescu C, et al. Mitral valve prolapse and sudden cardiac arrest in the community. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2016;13:498-503.

5. Herman AR, Cheung C, Gerull B, et al. Outcome of Apparently Unexplained Cardiac Arrest: Results From Investigation and Follow-Up of the Prospective Cardiac Arrest Survivors With Preserved Ejection Fraction Registry. *Circulation. Arrhythmia and electrophysiology.* 2016;9:e003619.

CONTRIBUTION OF AUTHORS

Three papers were prepared as part of this thesis. The student (WA) was first author on all, for which he was responsible for study design, data analysis and interpretation, and manuscript preparation and revision. One manuscript used data from the CASPER registry. Several colleagues contributed to this database and were offered authorship accordingly. In addition, the systematic review included in this thesis was completed through collaboration with other researchers. These researchers were offered authorship commensurate with their contributions. Drs. Wells, Nair, and Krahn provided guidance and feedback on all projects.

ACKNOWLEDGEMENTS

I am deeply grateful to the following colleagues without whom this work would not have been possible:

Andrew Krahn †

George A. Wells *†

Girish Nair *†

David Birnie

Martin Green

Ian Burwash

Omar Dewidar

Brianna Davies **

*Thesis supervisor

†Thesis advisory committee (TAC) members

** National coordinator for the CASPER registry

This work is dedicated to Rahaf and Talal

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

CHAPTER 3

LIST OF FIGURES

CHAPTER 2

CHAPTER 3

ABBREVIATIONS

MVP: Mitral valve prolapse

AMVP: Arrhythmic mitral valve prolapse

UCA: Unexplained cardiac arrest

IVF: Idiopathic ventricular fibrillation

SCD: sudden cardiac death

ICD: implantable cardioverter-defibrillator

ECG: electrocardiogram

Echo: echocardiogram

MR: mitral regurgitation

PVC: premature ventricular contraction

CMR: cardiac magnetic resonance imaging

ETT: exercise treadmill test

EPI: epinephrine challenge test

SCB: sodium channel blocker challenge test

BrS: brugada syndrome

CPVT: catecholaminergic polymorphic ventricular tachycardia

EPS: electrophysiology study

LQTS: long QT syndrome

CSP: coronary spasm provocation

FHx: family history

LGE: late gadolinium enhancement

TWI: T-wave inversion

ARVC: arrhythmogenic right ventricular cardiomyopathy

VA: ventricular arrhythmia

MAD: mitral annular disjunction

RV: right ventricle

CHAPTER 1: INTRODUCTION

1.1 Rationale

Mitral valve prolapse (MVP) has been reported to be a potential cause of cardiac arrest, even in the absence of left ventricular dysfunction or mitral regurgitation (MR). However, these reports are hindered by the incomplete evaluation to role out concealed causes of cardiac arrest due to the lack of a standardized evaluation of UCA patients. Although guidelines define IVF as cardiac arrest with cause identified despite "complete evaluation", they do not provide guidance as to what constitutes "complete evaluation", which led to inconsistency in reporting MVP as the only potential cause of cardiac arrest. Moreover, while the characteristics of patients with MVP as the only potential cause of cardiac arrest have been described before (i.e. characteristics of patients with arrhythmic MVP) , no studies have identified predictors of cardiac arrest that distinguish these patients from other MVP patients who have a benign prognosis.

1.2 Hypothesis

MVP is associated with IVF and has distinct features that can predict cardiac arrest.

1.3 Objectives

Our objectives were as follows:

1. To standardize the evaluation of UCA (cardiac arrest with no cause identified on coronary angiogram, ECG and Echo) and the definition of IVF (cardiac arrest with no cause identified despite extensive evaluation).
2. To determine the prevalence and characteristics of patients with MVP and IVF.

3. To determine predictors of cardiac arrest in patients with MVP.

1.4 Outline

This thesis has been prepared in a manuscript-based format. Three manuscripts are included (Chapters 3-5), in addition to a Background chapter (Chapter 2) and a Discussion chapter (Chapter 6).

1.5 Chapter summaries

Chapter 1: Introductory chapter detailing the structure of the thesis.

Chapter 2: Background information on the association between MVP and IVF, highlighting the importance of a standardized definition of IVF and the need to identify predictors of cardiac arrest in patients with MVP given its prevalence in the general population.

Chapter 3: Manuscript of a systematic review and meta-analysis of studies examining the yield of comprehensive cardiac evaluation in patients with UCA to develop a standardized evaluation of UCA and definition for IVF.

Chapter 4: Manuscript on the prevalence, characteristics and predictors AMVP, defined as MVP patients at risk for cardiac arrest. This manuscript used data from the CASPER registry and compared MVP patients with and without an alternative diagnosis.

Chapter 5: Protocol of a matched case-control study entitled: "Predicting Resuscitated Cardiac Cardiac Arrest with Echocardiographic and Doppler Images in Contemporarily Treated Mitral

Valve Prolapse (PREDICT-MVP)". This study is aiming at identifying and quantifying the association between pre-specified echocardiographic features and cardiac arrest in patients with MVP.

Chapter 7: Discussion on the overall results of the thesis and future directions.

CHAPTER 2: BACKGROUND

Cardiac arrest most often occur in patients with established heart disease such as coronary artery disease or cardiomyopathy.[1] However, it may develop in patients with no apparent cause on initial evaluation (i.e. UCA). UCA is a challenging diagnosis given the uncertainty about the prognosis and the difficulty of treating recurrent episodes of ventricular arrythmias in these patients.[2]

Finding a specific cause of UCA is an underappreciated step in the management of theses patients and has several important implications.[3] First, it guides choosing therapies to prevent and treat future arrythmias. Second, it guides assessing the risk of cardiac arrest in family members as some of these conditions are inherited. Last, it helps discover new conditions that carry a risk of cardiac arrest in the general population such as Brugada Syndrome (BrS), which was discovered during the work-up of UCA by the Brugada brothers.[4]

Multiple groups have published their results of comprehensive cardiac evaluations in patients with UCA and reported a high yield in finding the specific cause of UCA.[3, 5-7] However, only the minority of patients with UCA in real life undergo comprehensive cardiac evaluations.[3] Waldmann et al[3] reported that only 16% patients with UCA underwent comprehensive cardiac evaluation in Paris. This is likely, at least in part, due to the lack of a standardized approach to UCA endorsed by any guidelines.[8, 9] It is also important to note that some of tests used in some studies are expensive, invasive and/or not readily available. As such, defining the contribution of each test used to the overall yield is important. It is important to note that while the yield of a test (i.e. the proportion of patients diagnosed with a condition that is thought to be the cause of cardiac arrest out of all patients who underwent the test) is an important factor in determining what test need to be done, it does not take into account the accuracy of that test, which will need

to be tested separately. However, determining the yield of tests is an essential step that will help guideline developers and clinicians to choose what test to use and when to use it.

When the comprehensive cardiac evaluation fails to identify a cause for the UCA, patients are diagnosed with (IVF).[3, 5, 6] It is possible that this is not a separate clinical entity by itself but rather a combination of undiscovered conditions. Indeed, almost all conditions associated with UCA nowadays were labelled as IVF before that association was revealed.[4, 10] Developing a standardized definition of IVF will guide our continued efforts in examining conditions that are proposed to explain the cause of cardiac arrest in some patients with IVF such as MVP.

MVP is a common valvular abnormality with a prevalence of 1-3% in the general population.[11, 12] It has a benign prognosis overall however its association with sudden cardiac death (SCD) has been proposed.[13-15] The prevalence of MVP in patients with SCD or UCA has been reported in multiple studies.[14, 16, 17] Sriram et al[14] reported a prevalence of 42% in patients with UCA who underwent extensive evaluation with no identified cause. Basso et al[16] found that 7% of young adults with SCD and no structural heart disease on autopsy have MVP. On the other hand, in the population-based Oregon Sudden Unexpected Death Study, Narayanan et al[17] evaluated 729 patients with SCD and observed MVP in only 2.3%. The discrepancies in the reported prevalence of MVP in patients with SCD is likely due to the difference in population studied. It appears that MVP is only associated with cardiac arrest in those who underwent extensive evaluation to rule out other causes (i.e. IVF patients). However, there has not been a standardized definition for IVF used in these studies, which limits the ability to be certain about the association between MVP and IVF. It is also important to note that there are significant variations in demographics and referral biases among these studies that might contribute to the differences in the reported prevalence. This is suggested by the high degree of heterogeneity

(I^2=91.2%) reported in a recent meta-analysis that looked at the prevalence of MVP in unexplained SCD.[12]

It is probable that only a small subset of MVP patients is at high risk of SCD. Indeed, the reported annual risk of SCD in all MVP was found to be 0.2-0.4%, which is only slightly higher than the general population (≈ 0.1% per year).[12, 18] In addition, few reports have shown that certain subgroups of MVP patients are at substantially higher risk of SCD such as patients with flail leaflets or myxomatous changes.[15, 19, 20] The challenge is to identify this subset of patients early on to institute preventative therapies. Sriram et al[14] described the phenotype of patients with MVP and UCA and proposed the term "Malignant Bileaflet Mitral Valve Prolapse Syndrome" to identify this subset with high risk of SCD. Subsequently, an international study reported a similar phenotype with bileaflet prolapse, frequent premature ventricular contractions (PVCs) and female predominance.[21] However, none of these studies compared this phenotype in patients with MVP who did not have SCD. As such, it is unclear whether the features described in these patients can be used to identify patients at elevated risk of SCD.

Another important aspect of defining the prevalence and characteristics of MVP in UCA patients is having a well-characterized denominator. Studies that examined the relationship between MVP and SCD suffer from referral biases and the lack of a well-defined denominator.[14, 16, 21-24] CASPER registry is a prospective national registry that enroll UCA survivors and their first-degree relatives.[2] It provides the ideal set-up to answer the question of whether MVP is an important cause of UCA, given its multi-center nature and the extensive evaluation that patients undergo when enrolled. While it is unlikely that the causality between MVP and SCD will be concluded, better defining the prevalence and characteristics of MVP in patients with UCA is certainly an important step forward in the journey of interrogating the relationship between MVP

and SCD. Moreover, identifying unique features in MVP patients who survived UCA can help risk stratify patients with MVP in the general population. Multiple new echocardiographic and cardiac magnetic resonance findings such as mitral annular disjunction, lateral mitral annular velocities and late gadolinium enhancement have been proposed as predictors of arrhythmic events in MVP patients and these were not examined in the previous studies that reported the characteristics of patients with UCA and MVP.[25-27]. Moreover, the prevalence of these features in MVP patients in general is not well defined which challenges the use of these features to screen patients with MVP who might be at high risk of SCD.

References

1. Behr E, Wood DA, Wright M, et al. Cardiological assessment of first-degree relatives in sudden arrhythmic death syndrome. *Lancet.* 2003;362:1457-1459.

2. Krahn AD, Healey JS, Chauhan V, et al. Systematic assessment of patients with unexplained cardiac arrest: Cardiac Arrest Survivors With Preserved Ejection Fraction Registry (CASPER). *Circulation.* 2009;120:278-285.

3. Waldmann V, Bougouin W, Karam N, et al. Characteristics and clinical assessment of unexplained sudden cardiac arrest in the real-world setting: focus on idiopathic ventricular fibrillation. *European heart journal.* 2018;39:1981-1987.

4. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol.* 1992;20:1391-1396.

5. Herman AR, Cheung C, Gerull B, et al. Outcome of Apparently Unexplained Cardiac Arrest: Results From Investigation and Follow-Up of the Prospective Cardiac Arrest

Survivors With Preserved Ejection Fraction Registry. *Circulation. Arrhythmia and electrophysiology.* 2016;9:e003619.

6. Jimenez-Jaimez J, Peinado R, Grima EZ, et al. Diagnostic Approach to Unexplained Cardiac Arrest (from the FIVI-Gen Study). *Am J Cardiol.* 2015;116:894-899.

7. van der Werf C, Hofman N, Tan HL, et al. Diagnostic yield in sudden unexplained death and aborted cardiac arrest in the young: the experience of a tertiary referral center in The Netherlands. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2010;7:1383-1389.

8. Priori SG, Wilde AA, Horie M, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2013;10:1932-1963.

9. Al-Khatib SM, Stevenson WG, Ackerman MJ, et al. 2017 AHA/ACC/HRS Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death. *Circulation.* 2018;138:e272-e391.

10. Visser M, van der Heijden JF, Doevendans PA, Loh P, Wilde AA, Hassink RJ. Idiopathic Ventricular Fibrillation: The Struggle for Definition, Diagnosis, and Follow-Up. *Circulation. Arrhythmia and electrophysiology.* 2016;9.

11. Freed LA, Levy D, Levine RA, et al. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;341:1-7.

12. Nalliah CJ, Mahajan R, Elliott AD, et al. Mitral valve prolapse and sudden cardiac death: a systematic review and meta-analysis. *Heart.* 2019;105:144-151.

13. Miller MA, Dukkipati SR, Turagam M, Liao SL, Adams DH, Reddy VY. Arrhythmic Mitral Valve Prolapse: JACC Review Topic of the Week. *J Am Coll Cardiol.* 2018;72:2904-2914.

14. Sriram CS, Syed FF, Ferguson ME, et al. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. *J Am Coll Cardiol.* 2013;62:222-230.

15. Nishimura RA, McGoon MD, Shub C, Miller FA, Jr., Ilstrup DM, Tajik AJ. Echocardiographically documented mitral-valve prolapse. Long-term follow-up of 237 patients. *N Engl J Med.* 1985;313:1305-1309.

16. Basso C, Perazzolo Marra M, Rizzo S, et al. Arrhythmic Mitral Valve Prolapse and Sudden Cardiac Death. *Circulation.* 2015;132:556-566.

17. Narayanan K, Uy-Evanado A, Teodorescu C, et al. Mitral valve prolapse and sudden cardiac arrest in the community. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2016;13:498-503.

18. Deo R, Albert CM. Epidemiology and genetics of sudden cardiac death. *Circulation.* 2012;125:620-637.

19. Grigioni F, Enriquez-Sarano M, Ling LH, et al. Sudden death in mitral regurgitation due to flail leaflet. *J Am Coll Cardiol.* 1999;34:2078-2085.

20. Adabag AS, Therneau TM, Gersh BJ, Weston SA, Roger VL. Sudden death after myocardial infarction. *JAMA.* 2008;300:2022-2029.

21. Hourdain J, Clavel MA, Deharo JC, et al. Common Phenotype in Patients With Mitral Valve Prolapse Who Experienced Sudden Cardiac Death. *Circulation.* 2018;138:1067-1069.

22. Finocchiaro G, Papadakis M, Robertus JL, et al. Etiology of Sudden Death in Sports: Insights From a United Kingdom Regional Registry. *J Am Coll Cardiol.* 2016;67:2108-2115.

23. Naneix AL, Perier MC, Beganton F, Jouven X, Lorin de la Grandmaison G. Sudden adult death: an autopsy series of 534 cases with gender and control comparison. *J Forensic Leg Med.* 2015;32:10-15.

24. Meyer L, Stubbs B, Fahrenbruch C, et al. Incidence, causes, and survival trends from cardiovascular-related sudden cardiac arrest in children and young adults 0 to 35 years of age: a 30-year review. *Circulation.* 2012;126:1363-1372.

25. Perazzolo Marra M, Basso C, De Lazzari M, et al. Morphofunctional Abnormalities of Mitral Annulus and Arrhythmic Mitral Valve Prolapse. *Circ Cardiovasc Imaging.* 2016;9:e005030.

26. Kitkungvan D, Nabi F, Kim RJ, et al. Myocardial Fibrosis in Patients With Primary Mitral Regurgitation With and Without Prolapse. *J Am Coll Cardiol.* 2018;72:823-834.

27. Muthukumar L, Rahman F, Jan MF, et al. The Pickelhaube Sign: Novel Echocardiographic Risk Marker for Malignant Mitral Valve Prolapse Syndrome. *JACC Cardiovasc Imaging.* 2017;10:1078-1080.

CHAPTER 3: SYSTEMATIC REVIEW AND META-ANALYSIS OF STUDIES EXAMINING THE DIAGNOSTIC YIELD OF CARDIAC EVALUATION IN PATEINTS WITH UNEXPLAINED CARDIAC ARREST

The following is a manuscript with the objective of determining the yield of diagnostic testing in patients with UCA in order to develop a standardized definition of IVF that can be used in testing the association between any MVP and IVF (i.e. to ensure that no alternative causes of cardiac arrest were missed).

Short Title: Defining Idiopathic Ventricular Fibrillation

Authors: Wael Alqarawi MD[a,b], Omar Dewidar HBSc[a], Rafik Tadros MD PhD[c], Jason D. Roberts MD MAS[d], Christian Steinberg MD[e], Ciorsti J. MacIntyre MD[f], Zachary W. M. Laksman MD[g], Martin S. Green MD[b], Girish Nair MD MSc[a,b], George Wells PhD[a,h], Andrew D. Krahn MD[g]

This manuscript was submitted to HeartRhythm journal and is under review.

The student (WA) was first author, for which he was responsible for study design, data collection, data analysis and interpretation, and manuscript preparation and revision. Drs. Wells, Nair, and Krahn provided guidance and feedback on all stages of this project. Other co-authors contributed to data collection and writing the manuscript.

Title Defining Idiopathic Ventricular Fibrillation: A Systematic Review of Diagnostic Testing Yield in Apparently Unexplained Cardiac Arrest

Short Title: Defining Idiopathic Ventricular Fibrillation

Authors: Wael Alqarawi MD[a,b], Omar Dewidar HBSc[a], Rafik Tadros MD PhD[c], Jason D. Roberts MD MAS[d], Christian Steinberg MD[e], Ciorsti J. MacIntyre MD[f], Zachary W. M. Laksman MD[g], Martin S. Green MD[b], Girish Nair MD MSc[a,b], George Wells PhD[a,h], Andrew D. Krahn MD[g]

Affiliations: a) School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada
b) Division of Cardiology, University of Ottawa Heart Institute, Ottawa, Canada
c) Cardiovascular Genetics Center, Montreal Heart Institute, Faculty of Medicine, Université de Montréal, Montreal, Canada
d) Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, Western University, London, Canada
e) Institut Universitaire de Cardiologie et Pneumologie de Québec, Laval University, Quebec City, Canada
f) Dalhousie University and Queen Elizabeth II Health Sciences Centre, Halifax, Canada
g) Center for Cardiovascular Innovation, Division of Cardiology, University of British Columbia, Canada
h) Cardiovascular Research Methods Centre, University of Ottawa Heart Institute, Canada

Word Count: 5645

Funding: None

Disclosures: None

Acknowledgment: We thank **Sarah Visintini for her assistance with the systematic search and** Margaret Sampson for peer reviewing the MEDLINE search strategy.

Correspondence: Wael Alqarawi
University of Ottawa
40 Ruskin street,
Ottawa, ON, Canada.
K1Y 4W7
Phone: (613) 696 7402
E-mail: walqarawi@ottawaheart.ca

Abstract

Background: Idiopathic ventricular fibrillation (IVF) is diagnosed in patients with apparently unexplained cardiac arrest (UCA) after varying degrees of evaluation. This is largely due to the lack of a standardized approach to UCA.

Objectives: We sought to develop an evidence-based diagnostic algorithm for IVF by systematically examining the yield of diagnostic testing in UCA probands.

Method: Studies reporting the yield of diagnostic testing in UCA were identified in MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and conference abstracts. Their methodological quality was assessed by the National Institutes of Health (NIH) quality assessment tool. Meta-analyses were performed using the random effects model.

Results: A total of 21 studies were included. The pooled comprehensive diagnostic testing yield was 43% [95% CI (39% - 48%)] with no differences based on the protocol used (sequential vs discretionary). Lower yield was seen when only definite diagnoses by pre-specified criteria were used (32% vs 47%, p=0.15). Epinephrine challenge (when used to diagnose catecholaminergic polymorphic ventricular tachycardia), Holter monitoring and family screening were associated with low yield (<5%), whereas Cardiac MRI, exercise treadmill test and sodium-channel blocker challenge were associated with high yield (≥ 5%). Coronary spasm provocation, electrophysiology study and systematic genetic testing were reported to be abnormal in a high proportion of UCA probands (>10 %).

Conclusion: Based on a systematic review of the yield of diagnostic testing in UCA, we proposed a standardized approach to UCA and criteria to assess the strength of IVF diagnosis.

Keywords: diagnostic criteria, diagnostic algorithm, cardiac arrest, ventricular fibrillation

Introduction:

Apparently unexplained cardiac arrest (UCA) is diagnosed when patients presenting with cardiac arrest are found to have no obstructive coronary artery disease, normal left ventricular systolic function, and a baseline electrocardiogram (ECG) that does not identify the cause of the cardiac arrest. Finding the cause of UCA is important as it guides family screening and has therapeutic and prognostic implications for probands. Multiple studies have established the role of comprehensive diagnostic testing in revealing a concealed cause of UCA.[1-6] However, these studies used different protocols and reported different results. Consequently, current guidelines recommend performing comprehensive diagnostic testing in UCA probands and only diagnosing idiopathic ventricular fibrillation (IVF) when that fails to identify the cause, however, do not provide a clear guidance as to the panel of tests that need to be performed for the comprehensive diagnostic testing to be considered complete.[7] This has resulted in considerable inconsistency in the literature regarding the use of the term "IVF" to describe UCA probands who underwent some degree of diagnostic testing.[7] Inconsistency in using the term "IVF" renders interpreting the literature on the association between IVF and any condition such as MVP difficult to interpret. As such, there is a need to develop a standardized approach to UCA in order to facilitate a uniform diagnosis of IVF in these patients. Systematically reviewing the yield of diagnostic testing used in UCA is an essential step in developing such an approach because the probability of missing an alternative diagnosis for cardiac arrest and consequently falsely diagnosing IVF depends on the yield of tests that were not performed. Thus, we sought to perform a systematic review and meta-analysis of studies reporting the diagnostic testing yield in patients with UCA.

Methods:

Literature search

The reporting of this review conforms to PRISMA guidelines. See Supplementary Table S1 for the PRISMA checklist. A protocol was devised and registered in PROSPERO (ID: CRD42020189981). The literature search strategy was developed in consultation with a medical librarian. See Supplementary Figure S1 for the complete search strategy. In brief, we searched MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and performed a hand search for conference abstracts from relevant professional associations. The search strategy was peer-reviewed by a second librarian and was conducted on June-15-2020.

Eligibility criteria

Studies that reported the diagnostic outcomes of comprehensive diagnostic testing in patients with UCA that were 1-year-old or older were included. Patients younger than 1-year-old were excluded due to the substantial differences in the evaluation and causes of cardiac arrest. UCA was defined as cardiovascular collapse of presumed cardiac etiology with no cause identified during initial evaluation that included cardiac imaging (typically transthoracic echocardiogram), ischemia assessment and a 12-lead ECG. Comprehensive diagnostic testing was defined as performing all tests deemed by the treating team to be necessary to identify the cause of UCA (i.e. patients were diagnosed with IVF when comprehensive diagnostic testing was completed). The primary outcome was the proportion of patients who eventually were "diagnosed". Patients were considered "diagnosed" if the treating team identified a condition that was thought to explain the cardiac arrest. Studies that reported the diagnostic yield of a single test in UCA

patients were reported separately. When studies reported their updated results after the initial publication, the most recent results were used for the meta-analysis.

Study selection and data extraction

Articles were independently screened by 2 investigators (WA and OD). Disagreements were resolved by consensus. Pre-specified study details were collected in duplicates and discrepancies were resolved by consensus. Comprehensive diagnostic testing was defined as "sequential" when all patients received the same sequence of testing and "discretionary" when the testing was performed at the discretion of the treating team.

The diagnostic yield of each test was determined by calculating the proportion of patients who were "diagnosed" based on the result of that specific test (out of all patients who underwent the test). These proportions were obtained from studies reporting the yield of single tests and were calculated from studies reporting the yield of comprehensive testing when essential data were provided. "Systematic" use of a test was defined as offering a test for all patients, whereas "discretionary" use was defined as selective offering of a test at the discretion of the treating team.

Risk of bias

The methodological quality of all included manuscripts was assessed using the National Institutes of Health (NIH) quality assessment tool.[8] This was performed by 2 investigators independently (WA and OD) and discrepancies were resolved by consensus. Funnel plots were generated, and publication bias was assessed by visual inspection for plot asymmetry.

Statistical analysis

The proportions of patients who were "diagnosed" were pooled using random-effects model. Freeman-Tukey double arcsine transformation was used to stabilize the variances associated with extreme proportions reported in certain studies (i.e. 0%).[9] Statistical heterogeneity between studies was assessed using the I^2 statistic. High heterogeneity was defined as $I^2 \geq 75\%$ as per protocol and the source of high heterogeneity, when present, was examined through subgroup and sensitivity analyses. We performed pre-specified analyses as per protocol and exploratory analyses when there was a biologic (e.g. when there is a concern about false positive results) and/or methodologic reason (e.g. when there is a concern about selection bias with non-consecutive inclusion of patients). Exploratory analyses were specified when presented. Meta-analyses were performed using RevMan v5.4 (Cochrane Collaboration, Copenhagen, Denmark) and MedCalc Statistical Software version 19.4.1 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2020).

Results:

Study selection and characteristics

Our search strategy revealed 3376 citations. Of these, 41 were selected for full text review. A total of 21 studies met inclusion criteria and were included in the systematic review. Supplementary Figure S2 depicts the PRISMA flow diagram. Seven studies reported the yield of comprehensive diagnostic testing and 14 reported the yield of single tests.

Among studies that reported the yield of comprehensive diagnostic testing, 6 were manuscripts and 1 was an abstract. One study reflected a "real-world" setting, and the rest reflected the practice of referral centers. Two studies included adults alone, 1 was restricted to pediatrics cases

and the rest included all age groups, but predominantly involved adults. Only 2 studies performed sequential testing and the rest were discretionary. Table 1 summarizes studies that reported the yield of comprehensive diagnostic testing.

Among studies that reported the yield of single tests, 9 examined genetic testing and the rest examined either coronary spasm provocation (CSP, N=3), electrophysiology study (EPS, N=2) or sodium-channel blocker challenge (SCB, N=1). Six studies were manuscripts and the remaining 8 were abstracts. Supplementary Tables S2 and S3 summarize studies that reported the yield of single tests.

The yield of comprehensive diagnostic testing

The pooled yield of comprehensive diagnostic testing was 43% [95% CI (39% - 48%)] with no heterogeneity between studies ($I^2 = 0\%$). Figure 1 shows the forest plot of the pooled estimate. After baseline investigations that identified cardiac arrest survivors as UCA, cardiac MRI (CMR) was the most common test performed (279/413, 68%) followed by genetic testing (262/413, 63%). About half of UCA patients received at least one form of provocative testing for channelopathy [SCB, exercise treadmill test (ETT) and/or epinephrine challenge (Epi)].

There were no differences in the yield based on the age group included (43% for all age groups vs. 44% for adults vs. 48% for pediatrics, p=0.83). Similarly, there were no differences between studies that performed sequential versus discretionary testing (44% vs 43%, p=0.87) or between multi-center versus single-center studies (43% vs 44%, p=0.93). Exploratory analysis stratifying studies by the use of pre-specified criteria for the strength of diagnosis and including only patients with definite diagnoses showed a large difference in the yield, albeit not statistically significant (32% with pre-specified criteria vs 47% without, p=0.15). Another exploratory

analysis including only studies with consecutive patients to avoid referral bias revealed similar yield (44% vs 43%, p=0.88).

Diagnostic yield of provocative testing for channelopathy (SCB, ETT and Epi)

Figure 2 depicts forest plots of diagnostic yields of single tests. The pooled diagnostic yield of SCB was 8% [95% CI (3% - 13%)], with moderate heterogeneity between studies (I^2 = 45%) (Figure 2A). An exploratory subgroup analysis based on the agent used suggested a lower yield with procainamide as compared to flecainide or ajmaline, however, was not statistically significant (5% vs 10% vs 12%, p=0.34) (Supplementary Figure S3-B). Brugada syndrome (BrS) was more often diagnosed when SCB was performed systematically as compared to discretionarily (16% vs 5%, p=0.03). This trend persisted even when the study using procainamide was excluded from the discretionary group (16% vs 5%, p=0.08) (Supplementary Figure S3-A).

The pooled diagnostic yield of ETT was 9% [95% CI (5% - 13%)] with no heterogeneity between studies (I^2 = 0%) (Figure 2B). Exploratory subgroup analysis of systematic versus discretionary use of ETT showed no difference in the yield (9% vs 11%, p=0.76).

The pooled diagnostic yield of Epi was 9% [95% CI (5% - 13%)] with no heterogeneity between studies (I^2 = 0%) and no difference between systematic versus discretionary use (9% vs 9%, p=0.93) (Figure 2C). Given that Epi is used to diagnose LQTS and catecholaminergic polymorphic ventricular tachycardia (CPVT), and due to the concern about false positive rates for LQTS[10], an exploratory analysis restricting its diagnostic role to CPVT revealed a yield of 3% [95% CI (1% - 6%)] with no heterogeneity (I^2 = 0%).[10, 11]

Diagnostic yield of genetic testing

There was high heterogeneity between studies reporting the diagnostic yield of genetic testing (I^2 = 86%), which precluded pooling the estimate. On inspection of the forest plot, it was apparent that one study had a distinctly higher yield than other studies (Cunningham 2020). Cunningham et al[5] was the only study that exclusively included pediatric patients, whereas other studies included predominantly adults. A subgroup analysis based on inclusion age (pediatric vs. predominantly adult) was performed and showed a statistically significantly higher yield of genetic testing in pediatric patients (39% vs 7%, P<0.001). Only 1 study performed systematic genetic testing and reported no diagnoses based on the result of genetic testing performed on 31 probands. The pooled estimate of discretionary genetic testing in adults was found to be 11% [95% CI (4% - 22%)] with moderate heterogeneity (I^2 = 55%) (Figure 2D).

An exploratory analysis examining the proportion of patients with an actionable variant (likely pathogenic or pathogenic variant by the American College of Medical Genetics [ACMG] criteria)[12] revealed a significantly higher proportion when using broad panels (defined as $\geq$ 100 genes) versus limited panels (< 100 genes) (46% vs 12%, P <0.0001).This definition of panels was derived by inspecting the forest plot for studies with similar yields and was not pre-specified. Supplementary Figure S4 shows the forest plot for the proportion of patients with an actionable variant. Out of the 86 actionable variants reported, 51 (59%) were in cardiomyopathy genes and the remainder were in channelopathy genes. Variants details reported are included in Supplementary Table S3.

Diagnostic yield of other tests (CMR, Holter, EPS, CSP and family screening)

Pooling CMR diagnostic yield estimates was not possible due to high heterogeneity, which was resolved by stratifying studies based on inclusion criteria ("real-world" setting vs referral

centers) with a 33% yield in "real-world" setting versus 10% in referral centers (P<0.0001) (Figure 2E).

The pooled diagnostic yield of Holter monitoring was 6% [95% CI (2% - 12%)] with no heterogeneity ($I^2 = 0\%$). Of note, 5 cases in total were diagnosed based on Holter monitoring: 3 were probable diagnoses (2 LQTS and 1 short QT syndrome, where no additional evidence to support these diagnoses were found) and the other 2 cases were not reported. Due to the lack of criteria for diagnosing LQTS and SQTS on Holter monitoring, an exploratory analysis excluding these cases revealed a yield of 3% [95% CI (0.5% - 7%)] (Figure 2E).

Only one study reported the diagnostic yield of family screening and found it to be 4/28 (14%), however, only 1/28 (4%) was supported by genetic testing (a proband with negative evaluation including SCB, but a first degree relative who had flecainide-induced type 1 Brugada pattern; both tested positive for a pathogenic variant in SCN5A). The pooled proportions of patients with abnormal EPS and CSP were 23% [95% CI (8% - 43%)] and 20% [95% CI (5% - 34%)], respectively. Details of protocols used, definitions of abnormal results and diagnoses made are available in Supplementary Table S2.

Prevalence of conditions diagnosed

Conditions diagnosed after comprehensive diagnostic testing for each study are included in Table 1. LQTS was the most common condition diagnosed in most studies. Two studies reported the prevalence of coronary spasm and found it to be significantly different (9% vs 31%, p=0.002). Of note, the study that reported the high prevalence of CS performed CSP in 39/88 (44%) patients, whereas the use of CSP in the other study was not reported.

The prevalence of channelopathies (defined as LQTS, BrS or CPVT) was significantly higher in studies reflecting referral centers as compared to "real-world" settings (64% vs 8%, P<0.0001). Also, the prevalence of channelopathies was significantly higher in referral centers when CMR utilization was low (<50%), as compared to high use of CMR (> 50%) (80% vs 51%, P<0.002). Supplementary Figure S5 shows forest plots for the prevalence of channelopathies.

Risk of bias

Supplementary Figure S6 shows the quality assessment for all manuscripts included. All studies reporting the yield of comprehensive diagnostic testing were judged to have good quality, and their funnel plot shows no evidence of a publication bias (Supplementary Figure S7).

Discussion:

Examining the totality of experience evaluation apparently unexplained cardiac arrest, the yield of comprehensive diagnostic testing was found to be consistently high; however, there were significant differences between studies in the tests used and the conditions diagnosed. The results of our analysis suggest that a systematic use of CMR, ETT and SCB should be incorporated in the definition of IVF, due to their high yield. More evidence is needed to determine the role of EPS, CSP and systematic genetic testing

The significant differences in the conditions diagnosed after comprehensive diagnostic testing of UCA probands are probably related to their different baseline characteristics. However, inaccurate diagnoses due to false positive results could have contributed to this variation, which

is supported by multiple findings in our analysis. We found that the use of pre-specified criteria for diagnosis was associated with a markedly reduced yield of comprehensive diagnostic testing. In addition, we found a higher prevalence of BrS in studies that used SCB systematically, a higher prevalence of coronary spasm in the study that frequently used Ergonovine challenge and a higher prevalence of channelopathies in studies with low use of CMR. These findings are suggestive but certainly not definitive, and should raise a concern about inaccurate diagnoses in UCA probands, which is not surprising given the lack of 'gold standard' tests for many conditions associated with UCA. However, it enforces the need to review diagnoses regularly and emphasizes the need to establish a standardized, evidence-based evaluation for patients with UCA.

Current guidelines recommend comprehensive diagnostic testing in UCA patients, and define IVF by the exclusion of alternative causes, but do not specify a minimum requirement for testing. Defining a minimum requirement for testing is important for 3 reasons. First, it prevents incomplete evaluation in patients presenting with UCA. Waldmann et al showed that only 8% of patients diagnosed with IVF underwent ETT and 18% did not have CMR.[13] It is probable that these tests would have revealed alternative diagnoses; rendering the diagnosis of IVF inaccurate. Second, it provides a more uniform definition for IVF. The term "IVF" is used in the literature for patients who have had varying extent of evaluation. As we identify new conditions that could be associated with IVF, it becomes essential to have a well-defined cohort of IVF patients, or else any attempt at studying associations can be misleading. Indeed, when one reviews the evidence that supports the association between arrhythmic mitral valve prolapse (AMVP) and IVF, it becomes apparent that the prevalence of AMVP varies depending on the rigors to which

alternative causes have been excluded. The prevalence of AMVP has been reported to be 2% when no alternative causes were excluded, 7% when structural, but not electrical causes were excluded, and 41% if all alternative causes were excluded.[14-16] Last, having a well-defined cohort of patients with IVF facilitates a better description of their genetic background, prognosis and response to various therapies.

Based on the pooled diagnostic yield, one can divide the tests incorporated in the comprehensive diagnostic testing of UCA probands into 3 groups. First, tests with high yield such as CMR, ETT and SCB. These should constitute the minimum requirement for testing before diagnosing IVF. In addition, the probability of missing an alternative diagnosis (i.e. strength of IVF diagnosis) should be based on how many of these tests were not performed. This is especially helpful in standardizing the terminology used when reporting patients with UCA who underwent an incomplete evaluation that failed to identify the cause of UCA. Second, tests with low yield such Epi, Holter monitor and family screening. These tests should be performed on a discretionary basis, and withholding them should not preclude diagnosing IVF. Given the established high false positive results of Epi when used to diagnose LQTS, it should mainly be used as an alternative to ETT (e.g. if patient can not exercise).[10] Third, tests such as EPS, CPS and systematic genetic testing that require further study, given the uncertain clinical significance of the reported high rates of abnormal findings. Figure 3 shows a proposed standardized approach to UCA, and criteria for the strength of IVF diagnosis.

The significantly higher prevalence of BrS in studies that used SCB systematically is an interesting finding. It may suggest that some of these diagnoses are false positives, rather than exclusively secondary to an improved diagnostic yield. Systematic performance of any test will result in a higher prevalence of the disease tested, either because of over-diagnosis (i.e. false positive results) or under-diagnosis (i.e. missing the disease with discretionary testing). Given the established concern about the specificity of SCB, one may speculate that the higher prevalence of BrS with systematic SCB is, at least in part, due to false positive results.[17-19] Tadros et al reported that 8% of families who had a positive SCB test (with ajmaline) had alternative diagnoses or non-segregation of the SCB response and arrhythmias; suggestive of false positive results.[19] As such, clinicians should be cautious when diagnosing UCA probands with BrS based solely on the result of SCB, and these patients should be assessed by experienced teams in specialized clinics to exclude alternative diagnoses. Only one study in our systematic review used procainamide for SCB, thus it is difficult to provide any recommendations specific to its use. However, in the absence of access to alternate SCBs, procainamide should be performed in all UCA probands before diagnosing IVF until certain criteria are established that support withholding SCB without the risk of missing BrS.

The clinical significance of the high proportion of abnormal EPS, CSP and genetic testing in UCA is unclear. The ongoing EPS ARREST study (NCT03079414) is examining the clinical value of performing EPS in UCA survivors, and will help define its role in the evaluation. Careful interpretation of CSP results is warranted given the suspected overlap with other conditions such as early repolarization syndrome (ERS) and BrS.[20, 21] Lastly, concealed arrhythmogenic cardiomyopathies have been shown to be associated with UCA, and could

potentially explain the high proportion of pathogenic variants in cardiomyopathy genes, which needs further study.[22, 23]

Our systematic review has several limitations. First, it is probable that some true causes of UCA were masked by inaccurate diagnoses in some studies, given that the diagnostic evaluation is often concluded once any diagnosis is made. This likely explains why the overall yield of diagnostic testing is similar in all studies despite the concern about false positive results in some of them. While this could have affected the prevalence of conditions diagnosed in these studies, it is unlikely that it had a significant impact on the yield of each test used, because we examined each test separately and performed sensitivity analyses when there was a concern about false positive results. However, more studies are needed to assess diagnostic accuracy of each test used in our proposed algorithm; not withstanding the difficulty of doing so given the lack of a gold standard test for most of conditions associated with UCA. Second, some conditions associated with UCA require multiple tests to reach the diagnosis, and determining what test was essential for the diagnosis can be challenging. However, we relied on the authors' judgment and provided data on specific diagnoses, when possible. Third, our objective was to determine the yield of diagnostic testing in detecting a clinical diagnosis rather than abnormal findings. However, we reported abnormal findings when available (such as actionable variants with genetic testing and induction of sustained VT with EPS), but it is possible that some studies that reported abnormal findings in UCA (without a clinical diagnosis) were not included, as it was not the focus of this review. Similarly, studies that reported abnormal findings in IVF patients (rather than UCA) were not included because it is a different population, however, the distinction between UCA and IVF can be challenging given the lack of standardized definitions. As such,

we performed sensitivity analyses whenever the distinction was not clear. Fourth, patients were enrolled over a long time period, which could have affected the evaluation and diagnoses they received as our understanding of the appropriate diagnostic evaluation for UCA and associated conditions has changed significantly over time. Last, like any other diagnostic criteria, there are unavoidable arbitrary components such as the dichotomous categorization of the diagnostic yield (high vs low). While we strived to avoid bias in developing our proposed algorithm, there are undoubtfully components that are affected by our individual and collective opinions.

Conclusion:

Based on our systematic review, a comprehensive evaluation of an apparently unexplained cardiac arrest leads to a diagnosis in 43% of patients. We propose a standardized algorithm for the diagnostic evaluation of UCA, which can serve to assess the strength of an IVF diagnosis. This promises to facilitate a more uniform use of the term "IVF" in the literature. Given the imperfect features of each test and the corresponding potential for false positive results, careful ongoing review of diagnoses made in UCA probands is essential, ideally in specialized clinics. The high proportion of abnormal EPS, CPS and genetic testing in UCA patients is intriguing, and warrants further study.

Perspectives:

Competency in Medical Knowledge:

Cardiac MRI, Sodium-channel blocker challenge and exercise treadmill test are associated with high diagnostic yield and should be performed in all patients with UCA before diagnosing IVF.

Competency in Patient Care:

Patients with UCA should be managed by an experience team to avoid inaccurate diagnoses

Translational Outlook 1:

Guidelines should recommend a standardized approach to UCA to facilitate a uniform use of the term “IVF”

References:

1. Krahn AD, Healey JS, Chauhan V, et al. Systematic assessment of patients with unexplained cardiac arrest: Cardiac Arrest Survivors With Preserved Ejection Fraction Registry (CASPER). *Circulation.* 2009;120:278-285.

2. Jimenez-Jaimez J, Peinado R, Grima EZ, et al. Diagnostic Approach to Unexplained Cardiac Arrest (from the FIVI-Gen Study). *Am J Cardiol.* 2015;116:894-899.

3. Herman AR, Cheung C, Gerull B, et al. Outcome of Apparently Unexplained Cardiac Arrest: Results From Investigation and Follow-Up of the Prospective Cardiac Arrest Survivors With Preserved Ejection Fraction Registry. *Circulation. Arrhythmia and electrophysiology.* 2016;9:e003619.

4. Kumar S, Peters S, Thompson T, et al. Familial cardiological and targeted genetic evaluation: low yield in sudden unexplained death and high yield in unexplained cardiac arrest syndromes. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2013;10:1653-1660.

5. Cunningham T, Roston TM, Franciosi S, et al. Initially unexplained cardiac arrest in children and adolescents: A national experience from the Canadian Pediatric Heart Rhythm Network. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2020;17:975-981.

6. Stepien-Wojno M, Poninska J, Rydzanicz M, et al. Sudden cardiac arrest in patients without overt heart disease: a limited value of next generation sequencing. *Pol Arch Intern Med.* 2018;128:721-730.

7. Priori SG, Wilde AA, Horie M, et al. HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes:

document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2013;10:1932-1963.

8. Murad MH, Sultan S, Haffar S, Bazerbachi F. Methodological quality and synthesis of case series and case reports. *BMJ Evid Based Med.* 2018;23:60-63.

9. Freeman MF TJ. Transformations related to the angular and the square roo. *Ann Math Stats.* 1950;21:607-611.

10. Krahn AD, Healey JS, Chauhan VS, et al. Epinephrine infusion in the evaluation of unexplained cardiac arrest and familial sudden death: from the cardiac arrest survivors with preserved Ejection Fraction Registry. *Circulation. Arrhythmia and electrophysiology.* 2012;5:933-940.

11. Churet M, Luttoo K, Hocini M, Haissaguerre M, Sacher F, Duchateau J. Diagnostic reproducibility of epinephrine drug challenge interpretation in suspected long QT syndrome. *J Cardiovasc Electrophysiol.* 2019;30:896-901.

12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17:405-424.

13. Waldmann V, Bougouin W, Karam N, et al. Characteristics and clinical assessment of unexplained sudden cardiac arrest in the real-world setting: focus on idiopathic ventricular fibrillation. *European heart journal.* 2018;39:1981-1987.

14. Narayanan K, Uy-Evanado A, Teodorescu C, et al. Mitral valve prolapse and sudden cardiac arrest in the community. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2016;13:498-503.

15. Basso C, Perazzolo Marra M, Rizzo S, et al. Arrhythmic Mitral Valve Prolapse and Sudden Cardiac Death. *Circulation.* 2015;132:556-566.

16. Sriram CS, Syed FF, Ferguson ME, et al. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. *J Am Coll Cardiol.* 2013;62:222-230.

17. Hasdemir C, Payzin S, Kocabas U, et al. High prevalence of concealed Brugada syndrome in patients with atrioventricular nodal reentrant tachycardia. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2015;12:1584-1594.

18. Viskin S, Rosso R, Friedensohn L, Havakuk O, Wilde AA. Everybody has Brugada syndrome until proven otherwise? *Heart rhythm : the official journal of the Heart Rhythm Society.* 2015;12:1595-1598.

19. Tadros R, Nannenberg EA, Lieve KV, et al. Yield and Pitfalls of Ajmaline Testing in the Evaluation of Unexplained Cardiac Arrest and Sudden Unexplained Death: Single-Center Experience With 482 Families. *JACC Clin Electrophysiol.* 2017;3:1400-1408.

20. Kamakura T, Wada M, Ishibashi K, et al. Significance of Coronary Artery Spasm Diagnosis in Patients With Early Repolarization Syndrome. *J Am Heart Assoc.* 2018;7.

21. Komatsu M, Takahashi J, Fukuda K, et al. Usefulness of Testing for Coronary Artery Spasm and Programmed Ventricular Stimulation in Survivors of Out-of-Hospital Cardiac Arrest. *Circulation. Arrhythmia and electrophysiology.* 2016;9.

22. Ingles J, Bagnall RD, Yeates L, et al. Concealed Arrhythmogenic Right Ventricular Cardiomyopathy in Sudden Unexplained Cardiac Death Events. *Circ Genom Precis Med.* 2018;11:e002355.

23. Cheung CC, Davies B, Krahn AD. Letter by Cheung et al Regarding Article, "Concealed Arrhythmogenic Right Ventricular Cardiomyopathy in Sudden Unexplained Cardiac Death Events". *Circ Genom Precis Med.* 2019;12:e002447.

Table1: Characteristics of studies examining the yield of comprehensive diagnostic testing in apparently unexplained cardiac arrest

Study ID	Setting	Characteristics of UCA	Evaluation protocol	Tests performed	Proportion diagnosed	Conditions diagnosed	Characteristics of IVF
Herman 2016[1] Canada (manuscript)	Multi-center registry	41.5 yrs (14.7), 40% Females	Tests performed at the discretion of the treating team. pre-specified criteria for the strength of diagnosis was used.	CMR= 77% ETT=73% SCB= 66% Epi=68% Genetics=79%	81/200 (40.5%)	LQTS=22% BrS=9% CPVT=12% CMP=22% Other=35%	42.7 yrs (14.5), 39% Females
Jimenez-Jaimez 2015[2] Spain (manuscript)	Multi-center registry	39.8 yrs (15.7), 40% Females	Sequential testing. CMR followed by Epi and SCB followed by family screening followed by genetic testing. pre-specified criteria for the strength of diagnosis was used	CMR= 37% ETT=NA SCB= 100% Epi=100% Genetics=67%	18/35 (51.4%)	LQTS=17% BrS=39% CPVT=28% CMP=0% Other=16%	42.3 yrs (15.6), 47% Females
Waldman 2018[3] France (manuscript)	Multi-center, "real-world" setting. Only Adults	NA	Tests performed at the discretion of the treating team. No pre-specified criteria for the	CMR= 82% ETT=11% SCB= 27% Epi=15%	39/88 (44.3%)	LQTS=3% BrS=3% CPVT=3% CMP=49%,	48.7 yrs (14.8), 30.6% Females

			strength of diagnosis was used	Genetics=15%		Other=42%	
Cunningham 2020[4] Canada (manuscript)	Multi-center, retrospective case series, Only Pediatrics	Median 13.8 yrs (IQR 9-16), 61% Females	Tests performed at the discretion of the treating team. No pre-specified criteria for the strength of diagnosis was used	CMR= 57% ETT= NA SCB=NA Epi=NA Genetics=79%	20/42 (47.6%)	LQTS=30% BrS=0% CPVT=30% CMP=20% Other=20%	NA
Kumar 2013[5] Australia (manuscript)	Single center, retrospective case series of consecutive patients, Only adults	32 yrs (14), 51% Females	All underwent ETT. Other tests were performed at the discretion of the treating team. Genetic testing only performed if a clinical phenotype is proven or suspected. pre-specified criteria for the strength of diagnosis was used	CMR= NA ETT=89% SCB= 46% Epi=20% Genetics=NA	15/35 (42.9%)	LQTS=33% BrS=20% CPVT=7% CMP=13% Other=27%	NA
Peres 2020[6] France (abstract)	Single center, retrospective case series	NA	NA	NA	14/26 (53.8%)	NA	NA
Stepien-Wojno 2018[7]	Single center,	36.4 yrs (11.7),	Sequential testing. Genetic testing followed by family	CMR= 32%	17/44 (38.6%)	LQTS=53%	37.6 yrs (12.5),

Poland (manuscript)	prospective case series of consecutive patients	48%	screening followed by ETT and Holter then either CMR or SCB depending on clinical suspicion. pre-specified criteria for the strength of diagnosis was used.	ETT=NA SCB= 11% Epi=NA Genetics=70%	BrS=18% CPVT=6% CMP=0% Other=23%	40.7% Females

Data presented as mean (standard deviation) or number (percentages) unless otherwise specified. UCA: apparently unexplained cardiac arrest, IVF: idiopathic ventricular fibrillation, CMR: cardiac MRI, ETT: exercise treadmill test, SCB: sodium channel blocker challenge, Epi: Epinephrine challenge test, LQTS: long QT syndrome, BrS: Brugada syndrome, CPVT: catecholaminergic polymorphic ventricular tachycardia, CMP: cardiomyopathy. CMP defined as arrhythmogenic right ventricular cardiomyopathy (ARVC), dilated cardiomyopathy (DCM), myocarditis or hypertrophic cardiomyopathy (HCM)

References:

1. Herman AR, Cheung C, Gerull B, et al. Outcome of Apparently Unexplained Cardiac Arrest: Results From Investigation and Follow-Up of the Prospective Cardiac Arrest Survivors With Preserved Ejection Fraction Registry. *Circulation. Arrhythmia and electrophysiology.* 2016;9:e003619.

2. Jimenez-Jaimez J, Peinado R, Grima EZ, et al. Diagnostic Approach to Unexplained Cardiac Arrest (from the FIVI-Gen Study). *Am J Cardiol.* 2015;116:894-899.

3. Waldmann V, Bougouin W, Karam N, et al. Characteristics and clinical assessment of unexplained sudden cardiac arrest in the real-world setting: focus on idiopathic ventricular fibrillation. *European heart journal.* 2018;39:1981-1987.

4. Cunningham T, Roston TM, Franciosi S, et al. Initially unexplained cardiac arrest in children and adolescents: A national experience from the Canadian Pediatric Heart Rhythm Network. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2020;17:975-981.

5. Kumar S, Peters S, Thompson T, et al. Familial cardiological and targeted genetic evaluation: low yield in sudden unexplained death and high yield in unexplained cardiac arrest syndromes. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2013;10:1653-1660.

6. Peres N HJ, Maille B, Arregle F, Zabern M, Dognin N, Koutbi L, Franceschi F, Deharo J.C. Comprehensive assessment of unexplained sudden cardiac death and idiopathic ventricular fibrillation during the index hospitalization: A systematic thorough investigation to unmask specific etiology. *Archives of Cardiovascular Diseases Supplements.* 2020;12:106-107.

7. Stepien-Wojno M, Poninska J, Rydzanicz M, et al. Sudden cardiac arrest in patients without overt heart disease: a limited value of next generation sequencing. *Pol Arch Intern Med.* 2018;128:721-730.

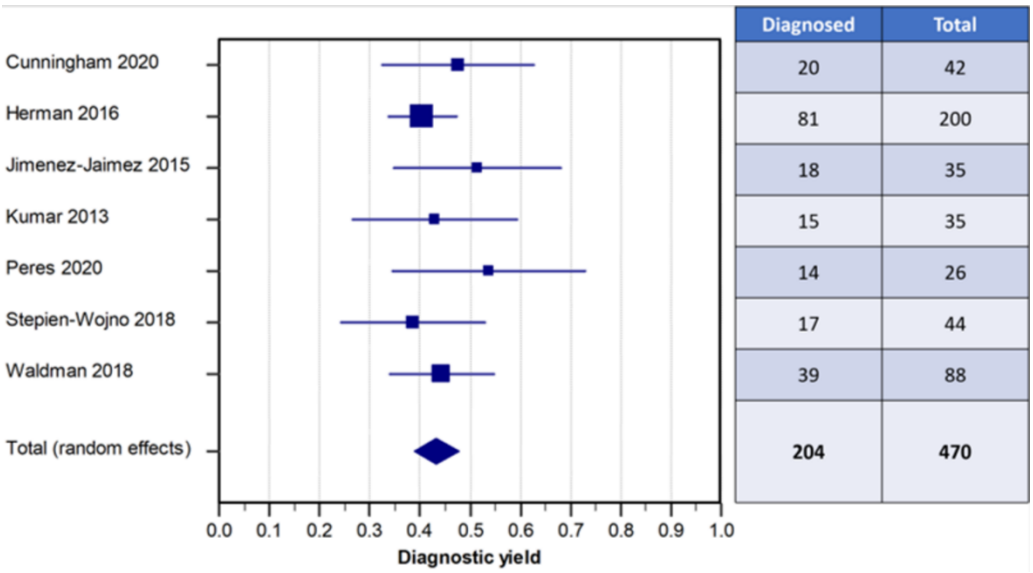


Figure 1: Forest plot of the yield of comprehensive diagnostic testing.

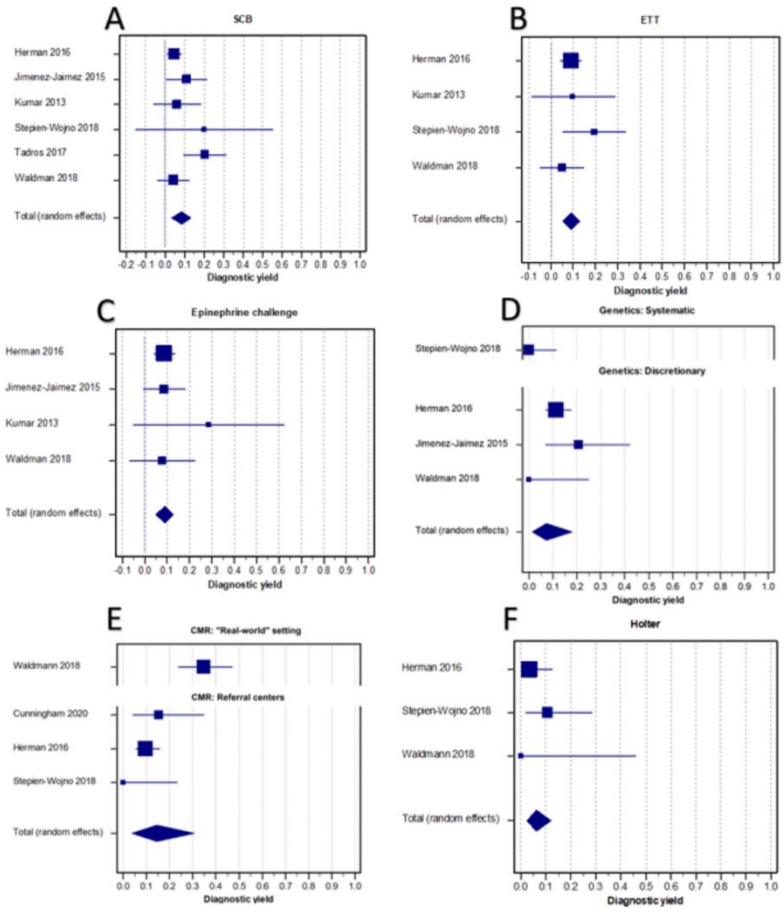


Figure 2: Forest plots of the diagnostic yield of single tests. Forest plots of meta-analyses of studies that reported the diagnostic yield of single tests. (A) SCB: sodium-channel blocker challenge. (B) ETT: exercise treadmill test. (C) Epinephrine challenge. (D) Genetic testing for adults, stratified by the protocol used (systematic vs discretionary). (E) CMR: cardiac MRI stratified by the setting (real-world vs referral centers). (F) Holter monitoring.

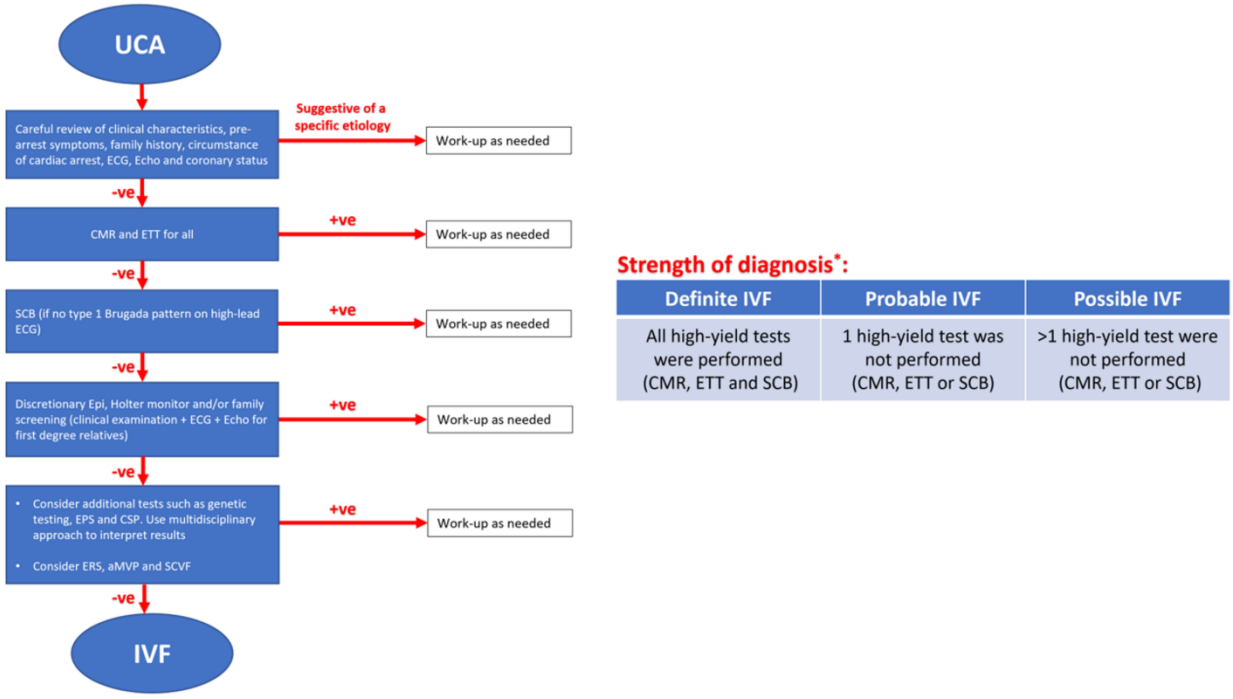


Figure 3: Suggested standardized approach to UCA and proposed criteria for the strength of IVF diagnosis.

* Adopted from Davies B et al. The Hearts in Rhythm Organization: A Canadian National Cardiogenetics Network. CJC Open (2020). aMVP: arrhythmic mitral valve prolapse, CMR: cardiac MRI, challenge, CSP: coronary spasm provocation, ECG: electrocardiogram, Echo: echocardiogram, Epi: Epinephrine challenge test, EPS: electrophysiology study, ERS: early repolarization syndrome, ETT: exercise-treadmill test, IVF: idiopathic ventricular fibrillation, SCB: sodium channel blocker, SCVF: short-coupled ventricular fibrillation, UCA: apparently unexplained cardiac arrest

Supplementary materials

Supplementary Table 1: PRISMA checklist

Supplementary Table 2: Summary of studies examining the yield of single diagnostic tests

Supplementary Table 3: Summary of studies examining the yield of genetic testing

Supplementary Figure 1: Search strategy

Supplementary Figure 2: Flow diagram for literature search

Supplementary Figure 3: Forest plots from subgroup analyses of the yield of sodium-channel blocker challenge. (A) Systematic versus discretionary use. (B) Procainamide versus Flecainide versus Ajmaline.

Supplementary Figure 4: Forest plots from subgroup analyses of the yield of genetic testing in detecting actionable variants (broad vs limited panel)

Supplementary Figure 5: Forest plots from subgroup analyses of the prevalence of channelopathies after comprehensive diagnostic testing. (A) prevalence of channelopathies in studies reflecting referral centers' setting versus "real-world" setting. (B) prevalence of channelopathies in studies with high versus low CMR use, defined as > 50% and $\leq$ 50%, respectively.

Supplementary Figure 6: A summary assessment of risk of bias for all studies included

Supplementary Figure 7: The funnel plot of studies that reported the yield of comprehensive diagnostic testing

Supplementary Table 1: PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a literature review.	Page 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings;	Page 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known about your topic.	Page 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Page 4
METHODS			
Eligibility criteria	5	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Page 5
Information sources	6	Describe all information sources (e.g., databases with dates of coverage) in the search and date last searched.	Page 5 and Online Figure 1
Search	7	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Online Figure 1
Study selection	8	State the process for selecting studies (i.e., screening, eligibility).	Pages 5 and 6
Risk of bias in individual studies	9	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level).	Page 4
Risk of bias across studies	10	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Page 6
RESULTS			
Study selection	11	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Page 7 and Online Figure 2

Section/topic	#	Checklist item	Reported on page #
Study characteristics	12	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1, Online Tables 2 and 3
Synthesis of results of individual studies	13	For all outcomes considered (benefits or harms), present, for each study: (a) summary of results and (b) relationship to other studies under review (e.g. agreements or disagreements in methods, sampling, data collection or findings).	Table 1 and Online Tables 2 and 3 for narrative review and Figures 1 and 2 and Online Figures 2-5 for meta-analyses
DISCUSSION			
Summary of evidence	14	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Pages 11 and 12
Limitations	15	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pages 15 and 16
CONCLUSION			
Conclusions	16	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Page 16

Adapted from: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews
and Meta-Analyses: The PRISMA statement. *PLoS Medicine*, 6(6), e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Supplementary Table 2: Summary of studies examining the yield of single diagnostic tests

Study ID	Protocol	Description of the test	Definition of a positive result	Proportion of positive tests	Proportion of clinical diagnosis
SCB					
Herman 2016[1]	Discretionary. Performed in 132/200 (66%) of UCA probands.	10 mg/kg Procainamide infusion for 20 minutes (to a maximum of 1 g)	> 1 mm ST-segment elevation (new or increase from baseline) in standard or high leads	NA	6/132 (4.6%)
Jimenez-Jaimez 2015[2]	Systematic. 35/35 (100%)	Flecainide used.	> 1 mm ST-segment elevation in standard or high leads	NA	4/35 (11%)
Waldman 2018[3]	Discretionary. Performed in 24/88 (27.3%) of UCA probands.	Ajmaline used.	NA	NA	1/24 (4.2%)
Kumar 2013[4]	Discretionary after ETT. Performed in 16/35 (45.7%) of UCA probands.	Flecainide used.	NA	NA	1/16 (6.3%)
Stepien-Wojno 2018[5]	Discretionary after genetic testing, family screening, ETT and Holter. Performed in 5/44 (11.4%) of UCA probands.	Flecainide used.	NA	NA	1/5 (20%)

Tadros 2017[6]	Performed in all UCA probands if BrS is suspected or can not be ruled out.	1 mg/kg Ajmaline infused at 10 mg/min. Higher doses were used in some cases.	ST-segment elevation >2 mm with a coved morphology in standard or high leads	11/54 (20.4%)	11/54 (20.4%)
ETT					
Herman 2016[1]	Discretionary. Performed in 146/200 of (73%) of UCA probands	Bruce or modified Bruce protocol. ECG performed at 1-minute interval during a 6-minute recovery phase	As per published algorithm[7] or induced ventricular arrhythmias during exercise	NA	13/146 (8.9%) LQTS and CPVT
Waldman 2018[3]	Discretionary. Performed in 10/88 of (11.3%) of UCA probands	NA	NA	NA	1/10 (10%) LQTS
Kumar 2013[4]	Systematic. Performed in 31/35 (88.6%) of UCA probands.	Sprint protocol with prolonged recovery up to 7 minutes	NA	NA	6/31 (19.4%) 3 LQTS 3 CPVT
Stepien-Wojno 2018[5]	Systematic. Part of a sequential testing that starts with genetic testing followed by family screening (ECG/Echo) followed by ETT. Performed in 20/44 (45.5%) of UCA probands.	Bruce protocol	Positive for LQTS if 4-minute recovery QTc is > 480 ms	NA	1/20 (5%) CPVT

Epi					
Herman 2016[1]	Discretionary. Performed in 136/200 of (68%) of UCA probands	Mayo clinic protocol[8]	For LQTS: if the absolute QT prolonged by ≥30 ms at 0.10 μg/kg per minute. For CPVT: if ≥3 beats of polymorphic VT or bidirectional VT	NA	12/136 (8.8%) LQTS and CPVT
Jimenez-Jaimez 2015[2]	Systematic. 35/35 (100%)	Mayo clinic protocol	Same as above	NA	3/35 (8.6%) 2 LQTS 1 CPVT
Waldman 2018[3]	Discretionary. Performed in 13/88 (14.8%) of UCA probands.	Mayo clinic protocol.	Same as above	NA	1/13 (7.7%) CPVT
Kumar 2013[4]	Discretionary after ETT. Performed in 7/35 (20%) of UCA probands.	NA	NA	NA	2/7 (28.6%) 2 LQTS
CMR					
Herman 2016[1]	Discretionary. Performed in 154/200 (77%) of UCA probands	NA	NA	NA	15/154 (10%)

Stepien-Wojno 2018[5]	Systematic. Part of a sequential testing that starts with genetic testing followed by family screening (ECG/Echo) followed by ETT. Performed in 14/44 (32%) of UCA probands.	NA	NA	NA	0/14 (0%)
Waldman 2018[3]	Discretionary. Performed in 72/88 (82%) of UCA probands.	NA	NA	NA	25/72 (35%)
Cunningham 2020[9]	Discretionary. Performed in 26/42 (62%) of UCA probands.	NA	NA	NA	4/26 (15%)
Holter					
Herman 2016[1]	Discretionary. Performed in 56/200 (28%) of UCA probands.	NA	NA	NA	2/56 (4%)
Stepien-Wojno 2018[5]	Systematic. Part of a sequential testing that starts with genetic testing followed by family screening followed by ETT and Holter. Performed in 28/44 (64%) of UCA probands.	NA	NA	NA	3/28 (11%) 2 probable LQTS and 1 probable SQTS
Waldman 2018[3]	Discretionary. Performed in 6/88 (7%) of UCA probands.	NA	NA	NA	0/6 (0%)

EPS					
Jimenez-Jaimez 2015[2]	Discretionary. Performed in 15/35 (43%)	NA	NA	NA	0/15 (0%)
Birnie 2012[10]	Discretionary in UCA patients.	Up to triple extra stimuli at 2 drive cycle lengths at each of two RV pacing sites.	Sustained VT	7/48 (15%)	NA
Peters 1998[11]	Systematic in IVF patients.	Up to triple extra stimuli at 2 drive cycle lengths at each of two RV pacing sites and long-short sequence of stimuli on and off Isoproterenol.	Sustained VT	6/18 (33%)	NA
CSP					
Waldmann 2018[3]	Discretionary. Performed in 39/88 (44%) of UCA probands.	Ergonovine	Transient coronary artery occlusion (>90% constriction) with angina and ischemic ECG.	12/39 (31%)	NA

Peters 1998[11]	Systematic in IVF patients.	Ergonovine administered for at 0.05 mg with increment Q3 min up to 0.45	≥ 1mm ST-segment elevation in ≥ 2 consecutive leads or induction of VT	1/18 (6%)	NA
Myerburg 1992[12]	Systematic in UCA patients	Ergonovine administered at 0.025 or 0.05 mg and up to 0.4	≥ 1mm ST-segment elevation or depression in ≥ 1 lead for at least 1 minute.	5/12 (42%)	NA
Meune 2003[13]	Systematic in IVF patients	Ergonovine administered at 0.05, 0.1 and 0.25 mg at 3 min interval	≥75% narrowing with chest pain and ≥ 2 mm ST-segment elevation in ≥ 2 consecutive leads	3/25 (12%)	NA
Family screening					
Herman 2016[1]	Discretionary. Performed in 65/200 (33%) of UCA probands.	NA	NA	14/85 (16%) 85 family members	NA
Jimenez-Jaimez 2015[2]	Systematic after CMR and provocative testing. Performed in 28/35 (80%) of UCA probands	ECG with provocative testing if borderline results and Echo	NA	NA	4/28 (14%) 3 BrS and 1 LQTS. All based on provocative testing. Only 1 supported by genetic testing

Cunningham 2020[9]	Discretionary. Performed in 26/42 (62%) of UCA probands.	NA	NA	6/26 (23%)	NA
Stepien-Wojno 2018[5]	Systematic. Performed in 32/44 (73%) of UCA probands.	ECG and Echo	NA	7/91 (8%) 91 family members	NA

Combined reference list attached to Supplementary Table S3. Data presented as number (percentages) unless otherwise specified. SCB: sodium channel blocker challenge, UCA: unexplained cardiac arrest, ETT: exercise treadmill test, BrS: Brugada syndrome, LQTS: long QT syndrome, CPVT: catecholaminergic polymorphic ventricular tachycardia, Epi: Epinephrine challenge test CMR: cardiac MRI. Echo: Echocardiogram.

Supplementary Table 3: Summary of studies examining the yield of genetic testing

Study ID	Protocol	Description of the test	Definition of actionable variants	Proportion of patients with an actionable variant	Proportion of clinical diagnosis
Herman 2016[1] (not included in the meta-analysis shown in Figure S4 due to overlap with Grondin 2019)	Discretionary after clinical testing was complete. Performed in 158/200 (79%) of UCA probands.	Direct sanger sequences and NGS of single and multiple gene panels.	NA	13/158 (8.2%) LQTS=2 CPVT=2 ARVC=7 SCN5A=2	18/158 (11.4%)
Jimenez-Jaimez 2015[2]	Patients with no phenotype identified on tests. Performed in 24/35 (67%) of UCA probands.	NGS of 126 genes.	NA	NA	5/24 (20.8%) 4 CPVT 1 SQTS
Waldman 2018[3]	Discretionary. Performed in 13/88 (15%) of UCA probands.	NA	NA	NA	0/13 (0%)
Cunningham 2020[9]	Discretionary. Performed in 33/42 (79%) of UCA probands.	NA	P/LP by 2015 ACMG classification	13/33 (39.4%) LQTS=3 CPVT=5 DCM=2 ARVC=1 Other=1	13/33 (39.4%)
Stepien-Wojno 2018[5]	Systematic. Performed in 31/44 (70%) of UCA probands.	NGS of 53 genes	P/LP by 2015 ACMG classification	2/31 (6.5%) 2 FLNC	0/31 (0%)

Mellor 2017[14] (not included in the meta-analysis shown in Figure S4 due to overlap with Grondin 2019)	Discretionary after clinical testing was complete. Performed in 174/375 (46%) of UCA probands.	Direct sanger sequences and NGS of single and multiple gene panels.	P/LP by 2015 ACMG classification	29/174 (16.7%) LQTS=9 CPVT=2 ARVC=8 SCN5A=6 DCM=3 HCM=1	NA
Asatryan 2018[15]	Systematic. UCA probands	NGS of 190 genes	P/LP by 2015 ACMG classification	7/31 (22.6%) P/LP > change to: 18/51	NA
Boo 2019[16]	Not defined	NGS of 174 genes	P/LP by 2015 ACMG classification	42/78 (53.8%) LQTS=3 SCN5A=7 ARVC=18 HCM=7 DCM=6 CACNA1C=1	NA
Giudicessi 2018[17]	Not defined	NGS of 32 genes	P/LP by 2015 ACMG classification	7/57 (12.3%) Cardiomyopathy=6 Channelopathy=1	NA
Leinonen 2018[18]	Systematic in UCA probands with negative phenotype despite extensive work-up	NGS of a mixture of 100 and 21 gene panels	NA	7/76 (9.2%) CPVT=5 ARVC=1 CACNA1C=1	NA

Mazzanti 2018[19]	Systematic in UCA probands with no KCNQ1, KCNH2, SCN5A and RYR2 mutations.	NGS of 50 gene	NA	3/30 (10%) ARVC=2 TRDN=1	NA
Probst 2015[20]	UCA	NGS of 163 genes	Putative mutation (not defined)	35/75 (46.7%)	NA
Visser 2017[21]	Systematic in UCA probands with negative phenotype despite extensive work-up	NGS of 33 genes followed by 179 genes if negative (33 patients underwent 179 genes)	Variants with a minor allele frequency of <0.05% were assessed for pathogenicity by using existing mutation databases and in silico predictive algorithms.	13/74 (17.6%) LQTS=1 CPVT=3 ARVC=4 DPP6=3 HCM=1 DCM=1	NA
Grondin 2019[22]	Systematic in UCA probands	NGS of 60 genes	P/LP by 2015 ACMG classification	30/234 (12.8%)	NA

Data presented as numbers (percentages). UCA: unexplained cardiac arrest, NGS: next generation sequencing, LQTS: long QT syndrome, CPVT: catecholaminergic polymorphic ventricular tachycardia, ARVC: arrhythmogenic right ventricular cardiomyopathy, SQTS: Short QT syndrome, P:pathogenic variant, LP: likely pathogenic variant, ACMG: American College of Medical Genetics and Genomics, DCM: dilated cardiomyopathy, HCM: hypertrophic cardiomyopathy, IVF: idiopathic ventricular fibrillation.

References:

1. Herman AR, Cheung C, Gerull B, et al. Outcome of Apparently Unexplained Cardiac Arrest: Results From Investigation and Follow-Up of the Prospective Cardiac Arrest Survivors With Preserved Ejection Fraction Registry. *Circulation. Arrhythmia and electrophysiology.* 2016;9:e003619.

2. Jimenez-Jaimez J, Peinado R, Grima EZ, et al. Diagnostic Approach to Unexplained Cardiac Arrest (from the FIVI-Gen Study). *Am J Cardiol.* 2015;116:894-899.

3. Waldmann V, Bougouin W, Karam N, et al. Characteristics and clinical assessment of unexplained sudden cardiac arrest in the real-world setting: focus on idiopathic ventricular fibrillation. *European heart journal.* 2018;39:1981-1987.

4. Kumar S, Peters S, Thompson T, et al. Familial cardiological and targeted genetic evaluation: low yield in sudden unexplained death and high yield in unexplained cardiac arrest syndromes. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2013;10:1653-1660.

5. Stepien-Wojno M, Poninska J, Rydzanicz M, et al. Sudden cardiac arrest in patients without overt heart disease: a limited value of next generation sequencing. *Pol Arch Intern Med.* 2018;128:721-730.

6. Tadros R, Nannenberg EA, Lieve KV, et al. Yield and Pitfalls of Ajmaline Testing in the Evaluation of Unexplained Cardiac Arrest and Sudden Unexplained Death: Single-Center Experience With 482 Families. *JACC Clin Electrophysiol.* 2017;3:1400-1408.

7. Sy RW, van der Werf C, Chattha IS, et al. Derivation and validation of a simple exercise-based algorithm for prediction of genetic testing in relatives of LQTS probands. *Circulation.* 2011;124:2187-2194.

8. Vyas H, Hejlik J, Ackerman MJ. Epinephrine QT stress testing in the evaluation of congenital long-QT syndrome: diagnostic accuracy of the paradoxical QT response. *Circulation.* 2006;113:1385-1392.

9. Cunningham T, Roston TM, Franciosi S, et al. Initially unexplained cardiac arrest in children and adolescents: A national experience from the Canadian Pediatric Heart Rhythm Network. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2020;17:975-981.

10. Birnie DH HJ, Gardner M, Chauhan V, Champage J, Sanatani S, Ahmed K, Gollob M, Simpson C, Skanes A, Gula L, Leong-Sit P, Yee R, Klein G, Krahn A. Role of electrophysiolgical testing in evaluation of patients with unexplained cardiac arrest. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2012; Volume9:S420-S438.

11. Peters RH, Wever EF, Hauer RN, Robles de Medina EO. Low prevalence of coronary artery spasm in patients with normal coronary angiograms and unexplained ventricular fibrillation. *European heart journal.* 1998;19:1070-1074.

12. Myerburg RJ, Kessler KM, Mallon SM, et al. Life-threatening ventricular arrhythmias in patients with silent myocardial ischemia due to coronary-artery spasm. *N Engl J Med.* 1992;326:1451-1455.

13. Meune C, Joly LM, Chiche JD, et al. Diagnosis and management of out-of-hospital cardiac arrest secondary to coronary artery spasm. *Resuscitation.* 2003;58:145-152.

14. Mellor G, Laksman ZWM, Tadros R, et al. Genetic Testing in the Evaluation of Unexplained Cardiac Arrest: From the CASPER (Cardiac Arrest Survivors With Preserved Ejection Fraction Registry). *Circ Cardiovasc Genet.* 2017;10.

15. Asatryan B, Seiler J, Servatius H, et al. P3817Diagnostic yield of genetic testing in cardiac arrest survivors with or without clinical evidence of cardiac disease: A swiss experience. *European heart journal.* 2018;39.

16. Boo KY KY, Oh S-K, Kim DY, Park H-S, Lee K-N, Shim J, Kim JS, Park S-W, Choi J-I, Kim Y-H,. Next-generation Sequencing Based Genetic Testing Can Detect Concealed Cardiomyopathies in Patients With Idiopathic Ventricular Fibrillation. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2019;16.

17. Giudicessi JR TD, Shehab O, Simpson M, Behr E, Ackerman MJ. Whole exome sequencing highlights the potential role of preclinical cardiomyopathies in the pathogenesis of sudden unexplained cardiac arrest and death in the young. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2018;15.

18. Leinonen JT, Crotti L, Djupsjobacka A, et al. The genetics underlying idiopathic ventricular fibrillation: A special role for catecholaminergic polymorphic ventricular tachycardia? *Int J Cardiol.* 2018;250:139-145.

19. Mazzanti A AN, Gambelli P, Memmi M, Morini M, Bloise R, Monteforte N, Napolitano C, Priori SG. Rare genetic variants and long-term follow-up in patients with idiopathic ventricular fibrillation. *Circulation* 2018;138.

20. Probst V LSS, Kyndt F, Schott J-J, Gourraud J-B, Sacher F, Mabo P, Karakachoff M, Bonnaud S, Violleau J, Marijon E, Dumas F, Cariou A, Baron E, Lindenbaum P, Jouven X, Redon R. Genetic screening identifies a high proportion of mutations

in patients with idiopathic ventricular fibrillation and sudden cardiac death. *Archives of Cardiovascular Diseases Supplements* 2015;7.

21. Visser M, Dooijes D, van der Smagt JJ, et al. Next-generation sequencing of a large gene panel in patients initially diagnosed with idiopathic ventricular fibrillation. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2017;14:1035-1040.

22. Steffany Grondin BD, Christopher C Cheung, Christian Steinberg, Jeffrey S. Healey, Martin S. Green, Shubhayan Sanatani, Paul Angaran, Henry Duff, Laura Arbour, Richard Leather, Christopher S. Simpson, Martin Gardner, Colette Seifer, Guillaume Lettre, Mario Talajic, Zachary W. Laksman, Jason D. Roberts, Andrew D. Krahn, Rafik Tadros. Systematic whole-exome sequencing in unexplained cardiac arrest: Results from the Canadian CASPER registry and biobank; (Abstract). *Presented at 69th Annual Meeting of The American Society of Human Genetics, October, 2019 in Boston, Massachusetts.* 2019.

Search Methods

The systematic review searches were created by a medical librarian (SV) in collaboration with the project lead (WA). The search was created in MEDLINE using a combination of key terms and index headings related to unexplained cardiac arrest and diagnosis, peer-reviewed by a second librarian and translated to the remaining bibliographic databases (see Supplemental Files for full Medline search).

The searches were conducted June 2020 in MEDLINE (Ovid MEDLINE(R) ALL 1946-), Embase (Ovid Embase Classic + Embase 1947-), and Cochrane Central Register of Controlled Trials (from inception). Searches were limited to the English language and animal studies were also excluded. Search results were exported to Covidence and dduplicates were eliminated using the platform's duplicate identification feature.

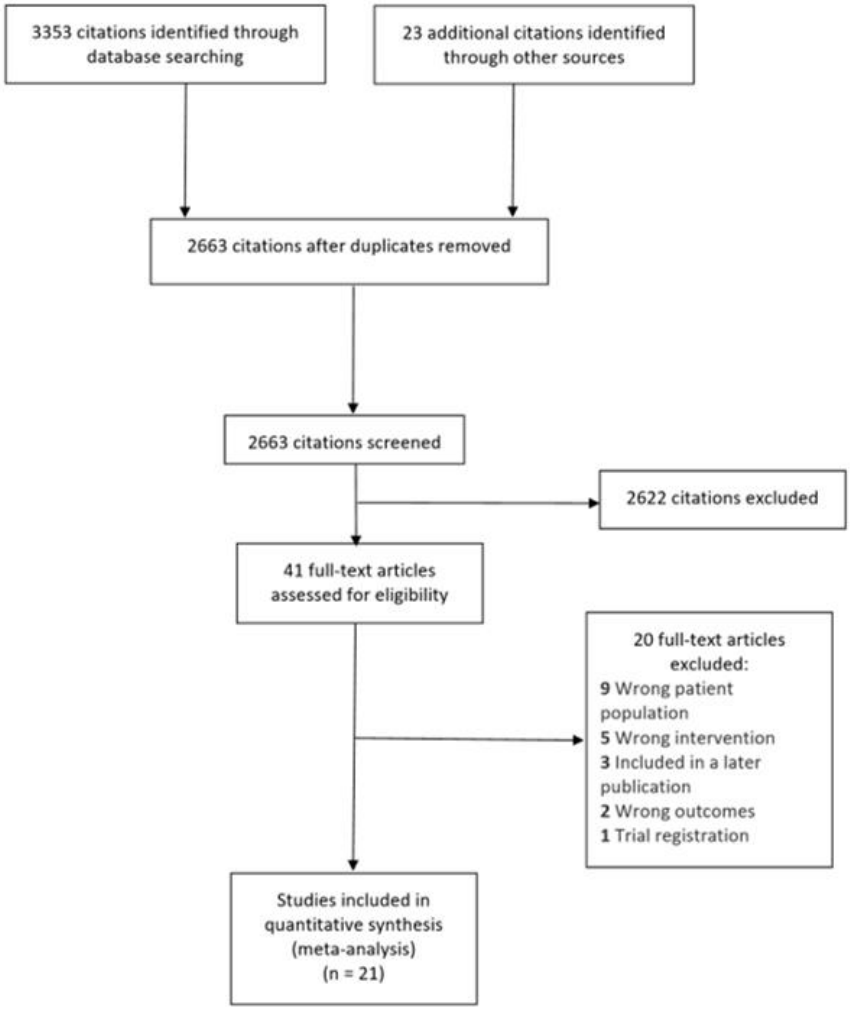
A hand search for conference abstracts not yet indexed in Embase was also conducted June 2020 for the following professional associations: American College of Cardiology, Heart Rhythm Society, American Heart Association, European Heart Rhythm Association and European Society of Cardiology Congress.

Supplemental Files

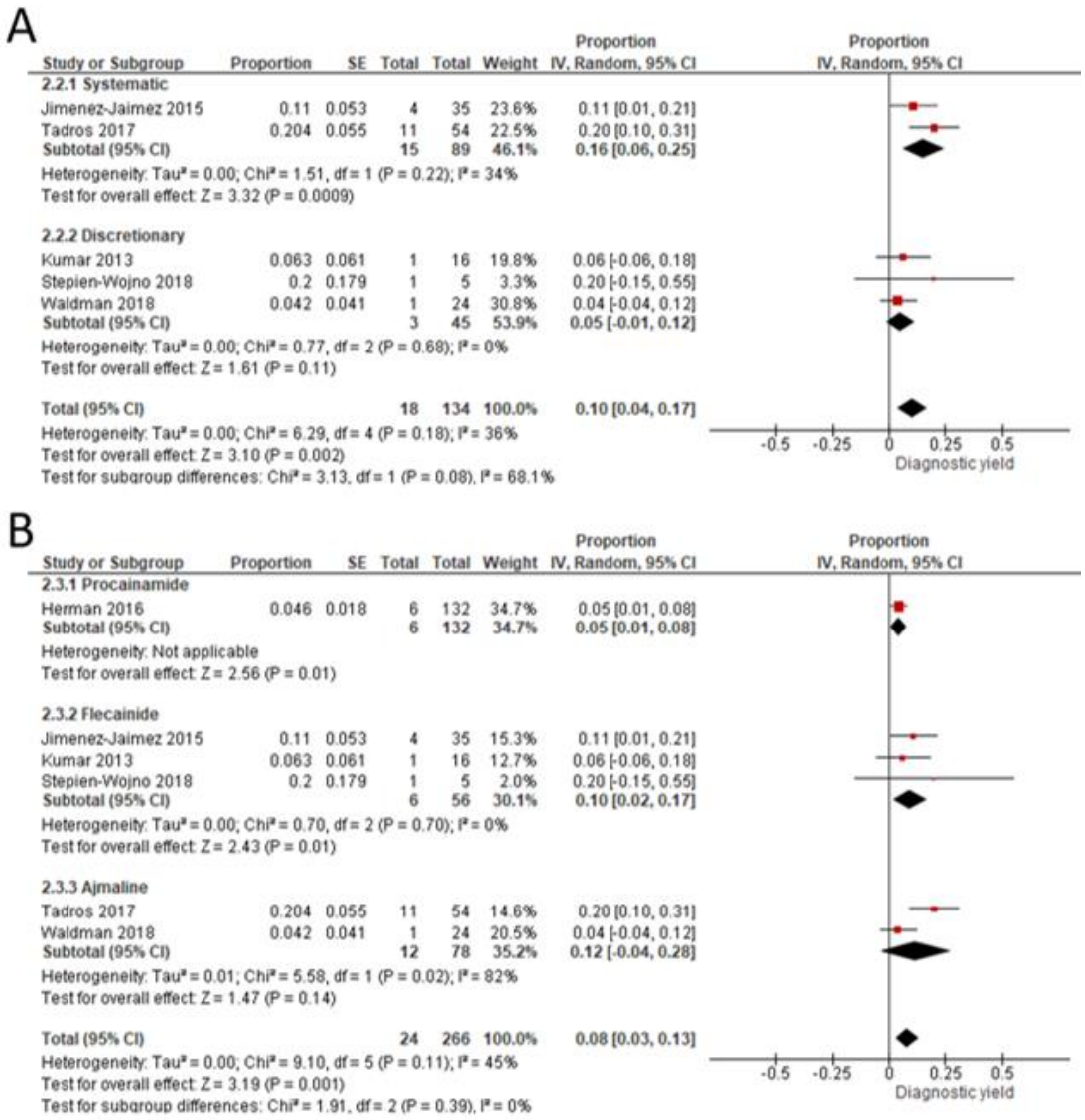
Table 1. Medline Search Strategy

#	Searches	Results
1	exp Heart Arrest/ and (unexplain* or idiopath* or silent).ti,ab,kf.	1053
2	((Unexplain* or idiopath* or silent*) adj3 (cardiac arrest or cardiac death)).ti,ab,kf.	178
3	((sudden adj2 unexplain* adj2 death) and (cardiologic* or cardiac)).ti,ab,kf.	381
4	((idiopath* or unexplain* or silent*) adj2 ventric* adj2 fibrill*).ti,ab,kf.	486
5	or/1-4	1638
6	(Etiology or diagnosis or pathology).fs.	6568159
7	(?etiolog* or diagnos* or test*).ti,ab.	5310220
8	(Comprehensiv* adj1 evaluat*).ti,ab.	8774
9	"Predictive Value of Tests"/	201251
10	exp Genetic Testing/	45570
11	exp Signal Processing, Computer-Assisted/	63854
12	exp Electrocardiography/	203400
13	exp Biopsy/	281104
14	((Famil* or genetic) adj2 (screen* or test*)).ti,ab.	45722
15	(SAECG? or high precordial lead* or ((signal-average* or high-lead or continuous) adj2 (ECG? or ekg? or electrocardiogra*))).ti,ab.	3439
16	(treadmill test* or ETT? or Exercise test* or stress test* or ecg challenge).ti,ab.	42783
17	((Epinephrine or channel-block* or sodium) adj2 challenge).ti,ab.	197
18	(CMR or ((cardiac or heart) adj31 (magnetic resonance imag* or MRI? or PET-MRI? or PETMRI?))).ti,ab.	23366
19	((heart or cardiac or myocardial or endomyocardial) adj3 biops*).ti,ab.	7178
20	(EPS or (electrophysiolg* adj1 (test* or study or evaluation or investigation))).ti,ab.	11504
21	(Ergonovine* or Acetylcholine* or ((coronary or spasm*) adj2 provoc*)).ti,ab.	100305
22	or/6-21	10203194
23	5 and 22	1401
24	exp animals/	23192210
25	exp animal experimentation/ or exp animal experiment/	9394
26	exp models animal/	564313
27	nonhuman/	0
28	exp vertebrate/ or exp vertebrates/	22535179
29	or/24-28	23194152
30	exp humans/	18490180
31	exp human experimentation/ or exp human experiment/	12440
32	30 or 31	18490834
33	29 not 32	4703942
34	23 not 33	1381
35	limit 34 to english language	1244

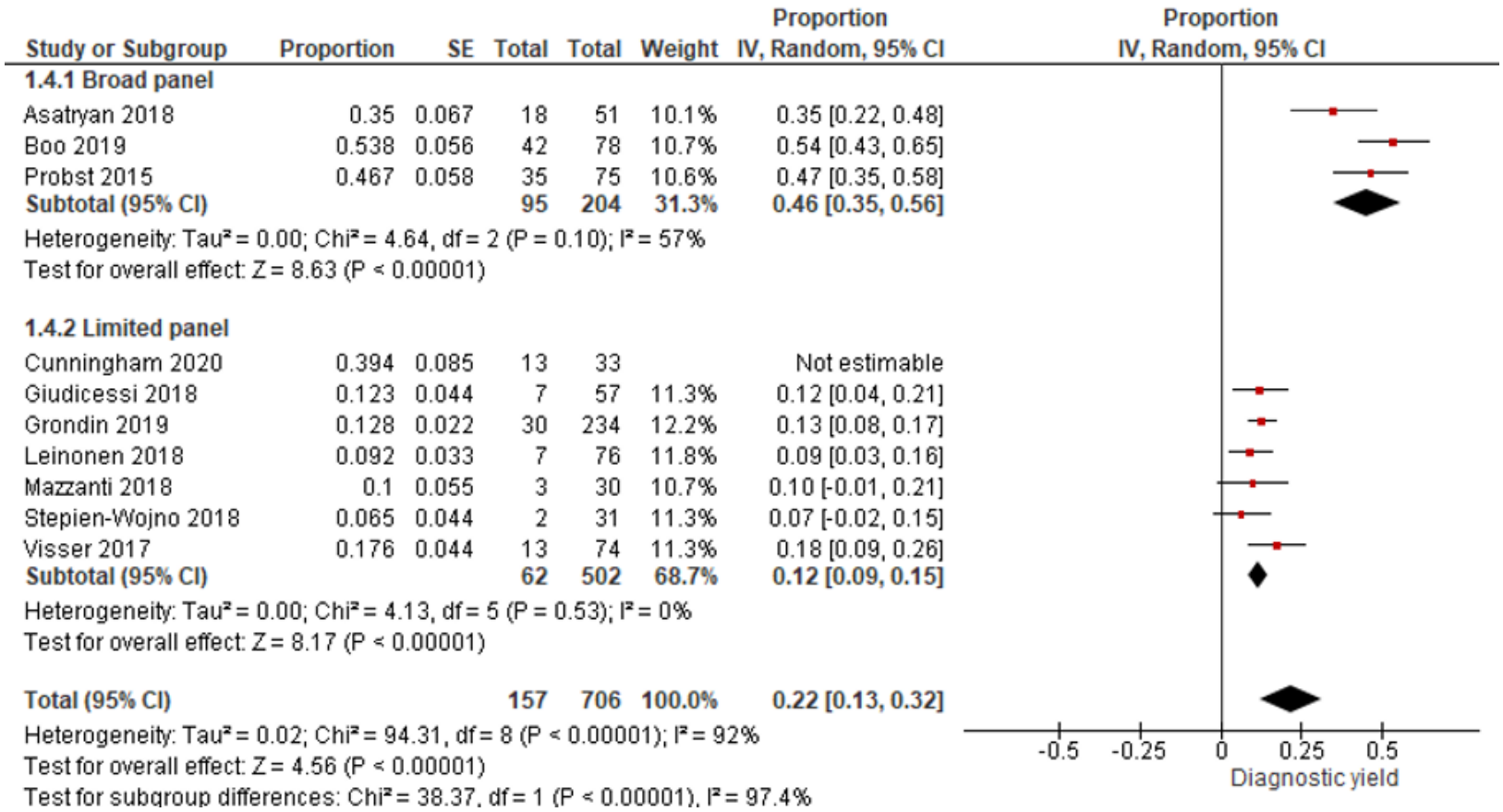
Supplementary Figure 1: Search strategy



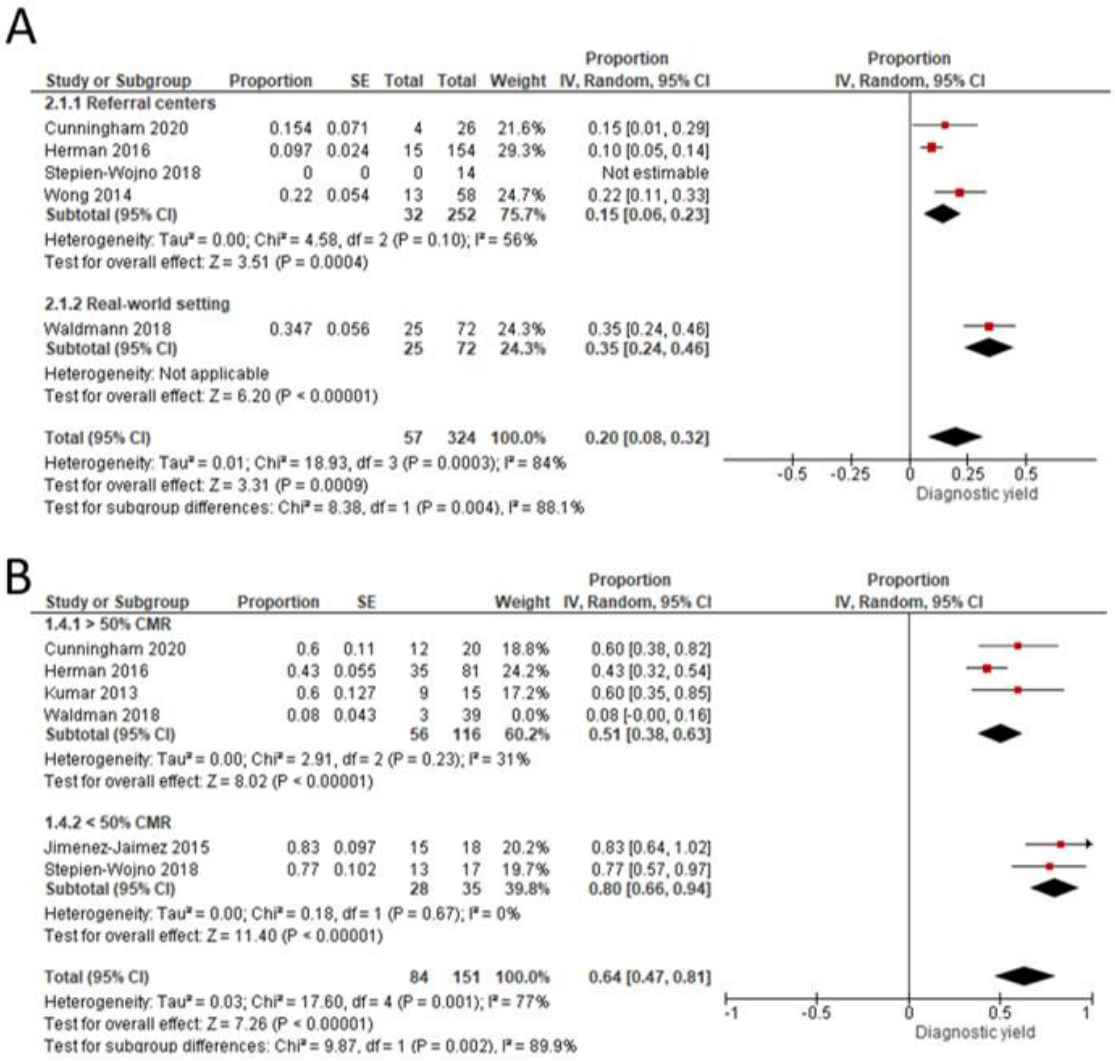
Supplementary Figure 2: Flow diagram for literature search



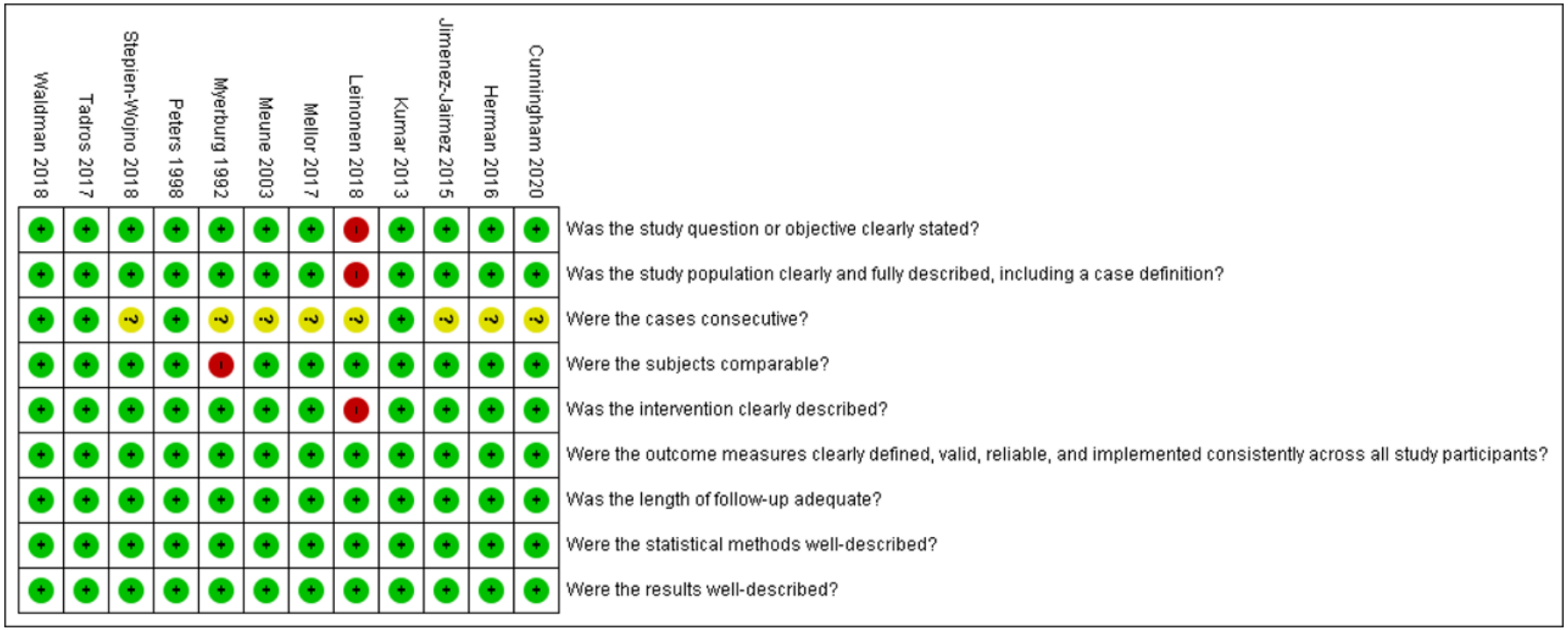
Supplementary Figure 3: Forest plots from subgroup analyses of the yield of sodium-channel blocker challenge. (A) Systematic versus discretionary use. (B) Procainamide versus Flecainide versus Ajmaline.



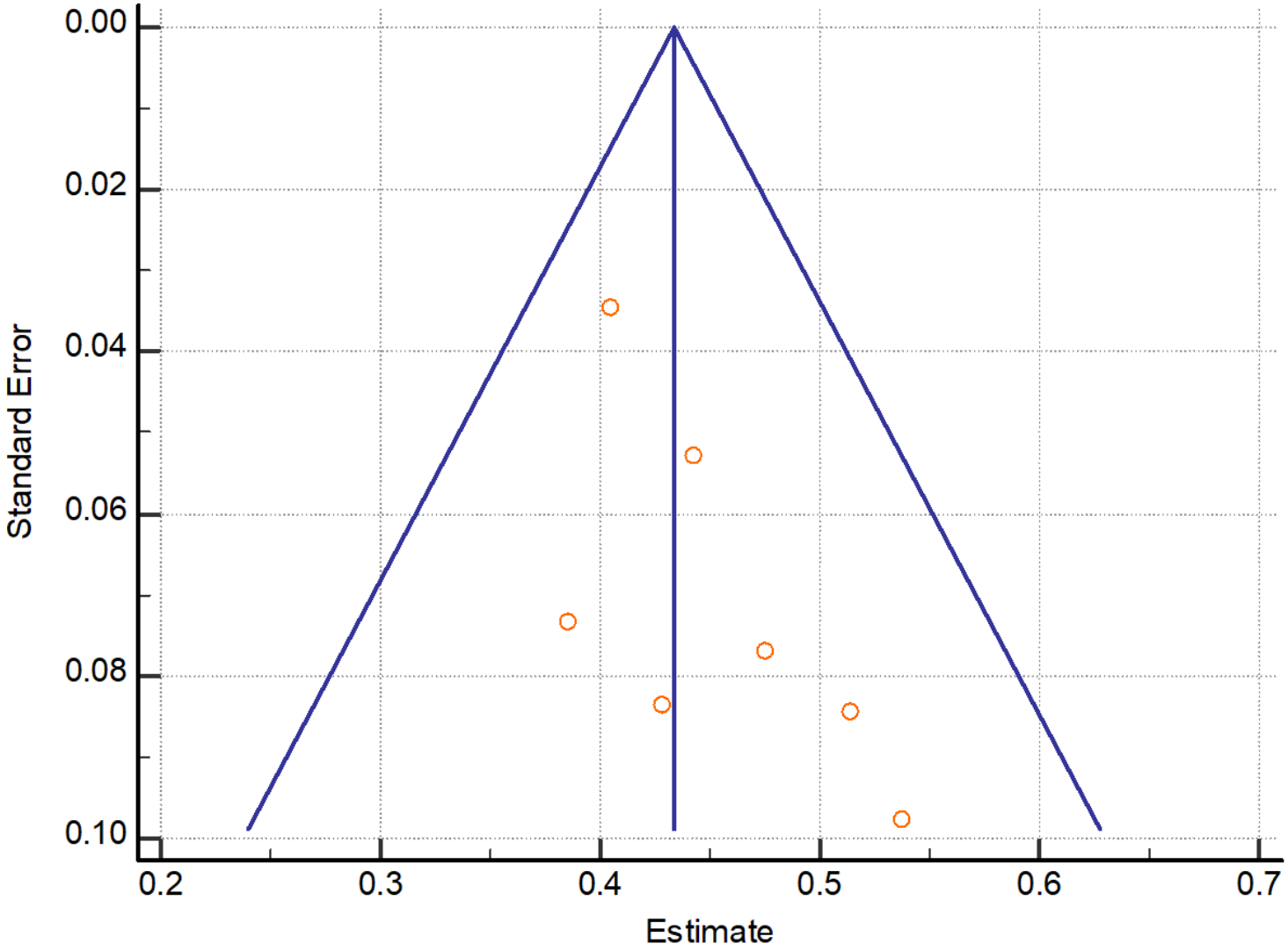
Supplementary Figure 4: Forest plots from subgroup analyses of the yield of genetic testing in detecting actionable variants (broad vs limited panel)



Supplementary Figure 5: Forest plots from subgroup analyses of the prevalence of channelopathies after comprehensive diagnostic testing. (A) prevalence of channelopathies in studies reflecting referral centers' setting versus "real-world" setting. (B) prevalence of channelopathies in studies with high versus low CMR use, defined as > 50% and ≤ 50%, respectively. CMR: cardiac MRI



Supplementary Figure 6: A summary assessment of risk of bias for all studies included



Supplementary Figure 7: The funnel plot of studies that reported the yield of comprehensive diagnostic testing

CHAPTER 4: THE PREVALENCE, CHARACTERISTICS AND PREDICTORS FOR ARRHYTHMIC MITRAL VALVE PROLAPSE: A REPORT FROM THE CASPER REGISTRY

The objective was to determine the prevalence, characteristics and predictors of AMVP, defined as MVP at risk for cardiac arrest. The proposed criteria in chapter 2 were used to test the association between MVP and IVF.

Short Title: Arrhythmic Mitral Valve Prolapse

Authors: Wael Alqarawi [a,b], Christopher C. Cheung [g], Girish Nair [b], Jason D. Roberts [d], Rafik Tadros [c], Martin S. Green [b], Jeffrey S. Healey [b], Christopher S. Simpson [b], Shubhayan Sanatani [g], Christian Steinberg [b], Martin Gardner [b], Ciorsti McIntyre [f], Paul Angaran [c], Henry Duff [c], Mario Talajic [c], Robert Hamilton [c], Laura Arbour [d], Richard Leather [d], Colette Seifer [d], Anne Fournier [e], Jacqueline Joza [e], George Klein [d], Zachary W.M. Laksman [g], George Wells [b,h], Andrew D. Krahn [g]

This manuscript is under preparation.

The student (WA) was first author, for which he was responsible for study design, data analysis and interpretation, and manuscript preparation and revision. Drs. Wells, Nair, and Krahn provided guidance and feedback on all stages of this project. Other co-authors contributed to data collection and writing the manuscript.

Title: Prevalence, Characteristics and Predictors of Arrhythmic Mitral Valve Prolapse: A Report from the CASPER Registry

Short Title: Arrhythmic Mitral Valve Prolapse

Authors: Wael Alqarawi [a,b], Christopher C. Cheung [g], Girish Nair [b], Jason D. Roberts [d], Rafik Tadros [c], Martin S. Green [b], Jeffrey S. Healey [b], Christopher S. Simpson [b], Shubhayan Sanatani [g], Christian Steinberg [b], Martin Gardner [b], Ciorsti McIntyre [f], Paul Angaran [c], Henry Duff [c], Mario Talajic [c], Robert Hamilton [c], Laura Arbour [d], Richard Leather [d], Colette Seifer [d], Anne Fournier [e], Jacqueline Joza [e], George Klein [d], Zachary W.M. Laksman [g], George Wells [b,h], Andrew D. Krahn [g]

Affiliations:

a) School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada

b) Division of Cardiology, University of Ottawa Heart Institute, Ottawa, Canada

c) Cardiovascular Genetics Center, Montreal Heart Institute, Faculty of Medicine, Université de Montréal, Montreal, Canada

d) Section of Cardiac Electrophysiology, Division of Cardiology, Department of Medicine, Western University, London, Canada

e) Institut Universitaire de Cardiologie et Pneumologie de Québec, Laval University, Quebec City, Canada

f) Dalhousie University and Queen Elizabeth II Health Sciences Centre, Halifax, Canada

g) Center for Cardiovascular Innovation, Division of Cardiology, University of British Columbia, Canada

h) Cardiovascular Research Methods Centre, University of Ottawa Heart Institute, Canada

Word Count: 3594

Correspondence: Wael Alqarawi
University of Ottawa
40 Ruskin street,
Ottawa, ON, Canada.
K1Y 4W7
Phone: (613) 696 7402
E-mail: walqarawi@ottawaheart.ca

Conflict of interest : The authors have no conflict of interest to disclose.

Abstract

Background: There is growing evidence that mitral valve prolapse (MVP) is associated with unexplained cardiac arrest (UCA). However, features distinguishing MVP patients at risk are lacking.

Objectives: To report the prevalence and characteristics of MVP patients who had UCA, and compare those with and without an alternative diagnosis.

Method: This was a retrospective cohort study utilizing data from CASPER registry which included patients with UCA, defined as cardiac arrest with no obstructive coronary artery disease, preserved left ventricular ejection fraction ($\geq$ 50%) and no apparent explanation on baseline electrocardiogram (ECG). A comprehensive standardized evaluation was performed, and patients were diagnosed with idiopathic ventricular fibrillation (IVF) if no cause was found. Echocardiography reports were systematically reviewed for MVP. Probable MVP was defined as "borderline/probable" prolapse and definite MVP was defined as "$\geq$ mild" prolapse. Patients with MVP were divided into 2 groups: those with IVF (Arrhythmic MVP, AMVP) and those with an alternative diagnosis for cardiac arrest (MVP+). Patient characteristics were compared between the 2 groups. The long-term outcomes of AMVP were reported and compared with IVF patients without MVP.

Results: Among 571 with UCA, 34 patients had MVP. The prevalence of definite MVP was significantly higher in patients with IVF than those with an alternative diagnosis [23/365 (6.3%) vs 5/206 (2.4%), P=0.045]. Bileaflet prolapse and family history (FHx) of sudden cardiac death (SCD) were more prevalent in AMVP, as compared with MVP+ [16/23 (70%) vs 3/10 (30%), P=0.0346] and [5/20 (25%) vs 0/9 (0%), P=0.099)], respectively. Higher proportion of AMVP patients received appropriate implantable cardiovertor-defibrillator therapies, as compared IVF patients without MVP [4/19 (21.1%) vs 28/268 (10.5%), P=0.156].

Conclusion: MVP appears to be associated with IVF. Bileaflet prolapse and FHx of SCD are more commonly seen in AMVP, as compared with MVP+. Further studies need to test the use of these features to identify AMVP in the general population.

Introduction:

Mitral valve prolapse (MVP) is a common valvular disease with a prevalence in the general population of 2.4%.[1] While most adverse cardiovascular outcomes in MVP are related to progressive mitral regurgitation (MR), sudden cardiac death (SCD) has been reported in patients without significant MR.[2] This has led to the interest in identifying the subset of patients with MVP who are at risk for malignant arrhythmic outcomes (i.e. Arrhythmic Mitral Valve Prolapse, AMVP).

To do so, Sriram et al[3] examined the prevalence and characteristics of MVP patients who survived cardiac arrest with no cause identified despite extensive evaluation (i.e. idiopathic ventricular fibrillation, IVF). This was the only study that examined the prevalence MVP in IVF and reported a substantially higher prevalence than the general population [42% (10/24)], suggesting an association between MVP and IVF. However, this was a single center study with small numbers and probably referral bias. In addition, while they described the common characteristics of these patients, which were later confirmed in an international collaborative study by Hourdain et al[4], no comparison was made between MVP patients with and without an alternative explanation for cardiac arrest. This is important to help distinguish characteristics that are specific to AMVP from those that are common in all MVP patients. Defining those characteristics will facilitate identifying patients with MVP who are at risk for malignant arrhythmias (i.e. AMVP) to institute primary prevention therapies such as implantable cardiovertor-defibrillator (ICDs).

As such, we sought to report the prevalence and characteristics of MVP patients in our national registry of unexplained cardiac arrest (UCA) and compare the characteristics of those with and without an alternative explanation for cardiac arrest.

Method:

Study population:

This study used data from the Cardiac Arrest Survivors with Preserved Ejection Fraction Registry (CASPER). Details of CASPER has been previously reported.[5] Briefly, CASPER is a national registry of UCA, defined as cardiac arrest with no obstructive coronary artery disease, preserved left ventricular ejection fraction ($\geq$ 50%) and no apparent explanation on baseline electrocardiogram (ECG). Enrollment started in 2004 with 4 centers and expanded to 20 centers that represent most centers that manage patients with UCA in Canada. Patients received standard testing to identify the cause of cardiac arrest including provocative testing, advanced imaging and genetic testing (Supplementary figure 1). Patient's characteristics, testing reports and follow-up data were prospectively captured in a secured database. Baseline and follow-up ECGs, exercise treadmill test (ETT) ECGs and Holter strips were retrospectively reviewed, when available, by experienced readers who were blinded to clinical data and basic pre-specified parameters were collected and entered in the database.

IVF:

Patients with no cause identified were diagnosed with IVF. For the purpose of this study, patients who had MVP with no alternative cause for cardiac arrest were included in this group and then described separately.

MVP:

All echocardiography (Echo) reports of patients enrolled in CASPER were screened for the presence of MVP. Probable MVP was defined as "borderline" or "probable" prolapse and definite MVP was defined as "≥ mild" prolapse. All patients were included, and a sensitivity analysis was then performed excluding patients with probable prolapse. Patients with MVP were categorized into 2 groups: MVP with an alternative diagnosis for cardiac arrest (MVP+) and MVP without an alternative diagnosis (i.e. AMVP: IVF patients with MVP). Echo and cardiac magnetic resonance (CMR) reports were reviewed to collect data such as location of prolapse, severity of MR and presence of late gadolinium enhancement (LGE). All available ECGs including ETT and Holter were reviewed by authors and data that was not captured by the pre-specified parameters included in the database was then added (T-wave inversion (TWI) in inferior leads, premature ventricular contraction (PVC) morphology, timing of PVCs during ETT and PVC burden on Holter).

Diagnostic strength of the cause of cardiac arrest:

All causes of cardiac arrest were categorized into possible, probable or definite diagnoses based on the certainty of the diagnosis, using established criteria when available (e.g. task force criteria for ARVC and Schawrtz score for LQTS). The full list of criteria was previously published

(Supplementary table 1). The cause of cardiac arrest was reviewed and updated at each follow-up visit. The strength of IVF diagnosis was also categorized into possible, probable or definite depending on the completeness of evaluation (i.e. probability of missing an alternative diagnosis), as recently proposed by our group (Supplementary figure 2). The strength of diagnosis was used to perform sensitivity analyses.

Follow-up

Patients were followed at each enrolling site, according to clinical standards. Follow-up data including appropriate Implantable Cardioverter-Defibrillator (ICD) therapies [shocks or anti-tachycardia pacing (ATP)] and death were recorded and uploaded in the database. Available ICD tracings were reviewed by authors and were described.

Statistical analysis:

Continuous variables were presented as mean ($\pm$ standard deviation) or median (interquartile range). Categorical variables were presented as absolute numbers (percentages). Student t-tests, Wilcoxon Rank-sum test, chi square tests, or Fisher's exact tests were used when appropriate to analyze data. A two-tailed p-value of <0.05 was considered significant for all comparisons. Kaplan-Meier survival curves for freedom from appropriate ICD therapies were generated and compared statistically using log-rank test. Analyses were performed using SAS (version *9.4*, The *SAS* institute, USA)

Results:

Overall cohort:

A total of 571 patients with UCA were included. Of those, 34 patients (6%) had MVP. In total, 206 patients were eventually found to have a diagnosis, rendering the yield of comprehensive evaluation of UCA to be 36.1% (206/571). This yield was not different between patients with or without MVP [10/34 (29.4%) vs 196/537 (36.5%), P=0.404].

IVF patients:

Table 1 summarizes the characteristics of patients with IVF and compare those with MVP (i.e. AMVP) versus those without MVP. The prevalence of MVP in IVF patients was found to be 6.6% [(24/365), 95% CI 4.5%-9.6%] which was not different when only patients with complete evaluation (i.e. definite IVF) were included (8/119, 6.7%).

The prevalence of MVP in IVF patients was higher but not statistically different than its prevalence in UCA patients who were eventually found to have an alternative cause for the cardiac arrest after evaluation [24/365 (6.6%) vs 10/206 (4.9%), P=0.404]. However, a sensitivity analysis including only patients with definite MVP showed significantly higher prevalence of MVP in IVF patients, as compared to those with an alternative diagnosis [23/365 (6.3%) vs 5/206 (2.4%), P=0.045].

The clinical and electrical characteristics of IVF patients with (i.e. AMVP) and without MVP were comparable, except for the significantly higher proportion of females in AMVP [16/24

(66.7%) vs 118/341 (34.6%), P=0.002]. Figure 1 depicts the prevalence of MVP in IVF and patients who were diagnosed.

MVP patients:

Table 2 shows the characteristics of AMVP, as compared to MVP patients with an alternative diagnosis for cardiac arrest (i.e. MVP+). Patients with AMVP had similar age and a comparable proportion of females. There were significantly more bileaflet prolapse in AMVP, as compared to MVP+ [16/23 (70%) vs 3/10 (30%), P=0.0346] without a significant difference in the severity of MR. Family history of SCD was seen more frequently in the AMVP than MVP+ group, albeit not statistically significant [5/20 (25%) vs 0/9 (0%), P=0.099). These observations remained the same with sensitivity analyses (when including only patients with definite alternative diagnoses and when including only patients with definite MVP), with the exception of family history of SCD, where no patients had a family history of SCD with a definite alternative diagnosis.

A detailed description of the evaluation and the final diagnosis of all patients with MVP included in the study is available in table 3. Notably, all patients with reported PVCs during ETT who had available tracings to determine PVC morphology had a morphology compatible with papillary muscle PVCs. Also, 2 patients (2/7, 28.6%) with available PVC burden on Holter had frequent PVCs (defined as $\geq$ 5%).[6] The first patient had AMVP with a burden of 10% (ID: 7171) and the other patient had possible CPVT with a burden of 12% (ID: 5859).

Follow-up:

A total of 287 patients with IVF had available follow-up data [19 with MVP (i.e. AMVP) and 268 without MVP]. The median follow-up was 56 months (IQR 26-96 months) with no significant difference between those with or without MVP (42 vs 57 months, P=0.299). Higher proportion of patients with MVP received appropriate ICD therapies as compared those without MVP, albeit not statistically significant [4/19 (21.1%) vs 28/268 (10.5%), P=0.156]. Consequently, the rate of ICD therapies was numerically higher in the MVP groups (0.3% per year vs 0.17% per year, respectively). Time to first appropriate ICD therapy is shown in the Kaplan Meier curves (Figure 2), demonstrating no statistically significant difference in freedom from appropriate ICD therapy between the two groups (Log rank P=0.657). One patient died at 40 months post arrest (ID:6969, IVF with no MVP and no history of appropriate ICD therapy).

Among AMVP patients, there were no differences in the characteristics of those who subsequently had an appropriate ICD therapy, except for a higher proportion of cardiac arrest during exercise [(2/4 (50%) vs 1/13 (7.7%), P=0.052)].

Discussion:

Our study described the prevalence and characteristics of MVP in a large cohort of patients with UCA. We observed 2 distinct phenotypes of MVP; one of which was associated with IVF (i.e. AMVP) and the other was likely an innocent bystander. We compared these 2 phenotypes and provided features that can potentially help distinguish AMVP patients from the large number of patients with MVP in the general population.

The prevalence of MVP in patients with IVF in our study was found to be 6.6%, which significantly lower than the 41% reported by Sriram et al, despite the similar evaluation.[3] This is likely related to the large sample size and the less potential for selection bias in our multicenter, national registry. In line with our results, Basso et al[7] reported the prevalence of MVP in a large registry of unexplained SCD to be 7% and Nalliah et al[2] meta-analyzed 18 studies the examined the prevalence of MVP in an otherwise unexplained SCD and found it to be 11.7%. The over-representation of MVP in patients with IVF, as compared to the general population, suggested an association between MVP and cardiac SCD, however, no previous study has shown this association. We found that the prevalence of definite MVP is significantly associated with IVF [23/364 (6.3%) vs 5/20 (2.5%), P=0.045], confirming the clinical observation that AMVP is likely a potential cause of IVF, not withstanding that association does not necessarily mean causation and more evidence is needed to prove causation.

Another important finding from our study is the yield of comprehensive evaluation of patients with MVP, which was not different than patients without MVP. This highlight the point that MVP should not be assumed to be the cause of cardiac arrest until proper evaluation is done to role out alternative causes, given the lack of features that distinguish AMVP from "bystander" MVP. While we can not rule out the contribution of MVP in cardiac arrest for patients who eventually were found to have an alternative diagnosis, the similar prevalence of MVP in these patients, as compared to the general population strongly suggest that MVP is indeed an innocent bystander.

Features that distinguish AMVP from MVP in general is not only important in deciding who would benefit from a comprehensive evaluation with UCA buy also, and more importantly, to identify AMVP patients prior to their cardiac arrest to institute primary prevention therapies such as ICD. The common phenotype of AMVP patients with female predominance, bileaflet prolapse, frequent complex ventricular arrythmias and inferior TWIs on ECG has been well described and is confirmed in our study. However, it was unclear from previous studies whether any or all of these features are common in MVP in general or specific to the subset of patients with AMVP. For example, the proportion of females in all case series of AMVP have been reported to be high but this is likely due to the fact that MVP, in general, is more common in females as shown in the large community-based cohort study and confirmed in ours.[1] As such, one should not use female gender as a feature to identify AMVP patients among MVP in general. In contrast, we showed for the first time that bileaflet prolapse is significantly associated with AMVP, which provides evidence supporting the clinical observation that bileaflet prolapse appears to distinguish the subset of patients with AMVP. We could not test other proposed features such ventricular arrhythmias, TWI and LGE in the current study given low numbers and the confounding effect of the underlying alternative cause of cardiac arrest (e.g. ventricular arrhythmias seen in patients with definite cardiomypathies and MVP bias the association toward the null). We did find however a new potentially promising feature which is family history of SCD that appears to be more prevalent in AMVP.

The novel finding of the differential prevalence of family history of SCD between MVP patients with and without an alternative cause for cardiac arrest is interesting and warrants a closer look.

Delling et al[8] reported the prevalence of family history of MVP in a large cohort of patients and found it to be 20.4%. This is interesting as it is similar to the prevalence of family history of SCD in our cohort of AMVP patients. Moreover, the international collaboration study that reported the characteristics of 42 patients with AMVP from 9 centers also reported a familial MVP of 14% and a prevalence of family history of SCD of 19%. The consistent finding of the high prevalence of family history of SCD and our finding of no family history of SCD in MVP patients with an alternative diagnosis suggest that this might be a feature that can distinguish the 2 phenotypes. One would not expect this high prevalence of family history of SCD in the general population with MVP. Clearly, however, future studies will need to confirm this observation and identify genetic determinants.

The long-term outcomes of AMVP patients showed that a significant proportion had recurrent ventricular arrhythmias (VAs) requiring ICD therapies. This calls for instituting preventative therapies to prevent VAs. However, while multiple therapies have been shown to reduce the risk of VAs in patients with AMVP such as catheter ablation and mitral repair, these are invasive procedures and will require further testing before their use for this indication.[9-11] The only predictor of recurrent ICD therapies in our study was the cardiac arrest with exercise, and the clinical significance of that will need to be explored in later studies.

Our study has several limitations. First, it is possible that there are cases with AMVP and cardiac arrest that were not included in the registry, given the potential explanation for their arrest on baseline Echo. However, the recognition of this condition as a cause of UCA is only recent and

we did not see any drop in enrolment of patients with MVP since its description [the median (IQR) date of enrolment/arrest in the overall cohort in patients with or without MVP: 2014 (2011-2016) and 2012 (2008-2015), respectively]. Second, there was no core lab to diagnose MVP on Echo, which can lead to over or under diagnosis. We are in the process of performing blinded reviews of these Echos to adjudicate the presence of MVP. In addition, for the current analysis, we performed a sensitivity analysis for questionable diagnoses (i.e. probable MVP) and reported results separately. Third, it is possible that MVP was the cause or, at least, a contributor to the cardiac arrest in patients who were found to have an alternative diagnosis. However, we performed sensitivity analyses including only definite diagnoses, based on our strict pre-specified criteria which showed similar results. It would be unlikely that a patient will have 2 causes of cardiac arrest especially given how rare these cause are in the general population. Also, one should expect MVP to be present in patients who had cardiac arrest for different reasons with a similar prevalence to the MVP in the general population, which was we precisely found in our study; albeit in a sensitivity analysis of definite MVP. This will be confirmed with blindly reviwing all Echos. Last, only bileaflet prolapse was statistically significantly different between AMVP and MVP+. However, once echos are reviewed, we are expecting to find multiple other echo parameters from our pre-specified list (supplementary Figure 3) to distinguish AMVP.

Conclusion:

MVP is not an uncommon finding in patients with UCA. Care should be taken before assigning the cause of cardiac arrest to MVP without careful evaluation to rule out alternative diagnoses. Some features appear to be more prevalent in patients with AMVP than patients with MVP+ such as bileaflet prolapse and family history of SCD. These need to be examined in future

studies and can potentially be used to identify patients with MVP who are at risk for malignant for arrhythmias in the general population.

References:

1. Freed LA, Levy D, Levine RA, et al. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;341:1-7.

2. Nalliah CJ, Mahajan R, Elliott AD, et al. Mitral valve prolapse and sudden cardiac death: a systematic review and meta-analysis. *Heart.* 2019;105:144-151.

3. Sriram CS, Syed FF, Ferguson ME, et al. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. *J Am Coll Cardiol.* 2013;62:222-230.

4. Hourdain J, Clavel MA, Deharo JC, et al. Common Phenotype in Patients With Mitral Valve Prolapse Who Experienced Sudden Cardiac Death. *Circulation.* 2018;138:1067-1069.

5. Herman AR, Cheung C, Gerull B, et al. Outcome of Apparently Unexplained Cardiac Arrest: Results From Investigation and Follow-Up of the Prospective Cardiac Arrest Survivors With Preserved Ejection Fraction Registry. *Circulation. Arrhythmia and electrophysiology.* 2016;9:e003619.

6. Dukes JW, Dewland TA, Vittinghoff E, et al. Ventricular Ectopy as a Predictor of Heart Failure and Death. *J Am Coll Cardiol.* 2015;66:101-109.

7. Basso C, Perazzolo Marra M, Rizzo S, et al. Arrhythmic Mitral Valve Prolapse and Sudden Cardiac Death. *Circulation.* 2015;132:556-566.

8. Delling FN, Rong J, Larson MG, et al. Familial clustering of mitral valve prolapse in the community. *Circulation.* 2015;131:263-268.

9. Syed FF, Ackerman MJ, McLeod CJ, et al. Sites of Successful Ventricular Fibrillation Ablation in Bileaflet Mitral Valve Prolapse Syndrome. *Circulation. Arrhythmia and electrophysiology.* 2016;9.

10. Vaidya VR, DeSimone CV, Damle N, et al. Reduction in malignant ventricular arrhythmia and appropriate shocks following surgical correction of bileaflet mitral valve prolapse. *J Interv Card Electrophysiol.* 2016;46:137-143.

11. Alqarawi W, Birnie DH, Burwash IG. Mitral valve repair results in suppression of ventricular arrhythmias and normalization of repolarization abnormalities in mitral valve prolapse. *HeartRhythm Case Rep.* 2018;4:191-194.

Table 1: Characteristics of patients with idiopathic ventricular fibrillation (IVF):

	IVF (n= 365)	AMVP (n=24)	No MVP (n=341)	P value
Age (years)	42 (31-52)	43.5 (35.5-49)	42 (31-53)	0.968
Gender (female)	134 (36.7%)	16 (66.7%)	118 (34.6%)	0.002
Ethnicity (white)	284 (77.8%)	18 (75%)	266 (78%)	0.795
Circumstance of death (exercise)	40/221 (18.1%)	3/17 (17.7%)	37/204 (18.1%)	0.96
Date of arrest (year)	2013 (2009-2016)	2014 (2011-2017)	2013 (2009-2016)	0.139
CMR performed	279 (77.3%)	20 (83.3%)	259 (76.9%)	0.584
ETT performed	247 (68%)	15 (62.5%)	232 (68.4%)	0.206
Procainamide performed	192 (52.6%)	12 (50%)	180 (52.8%)	0.957
FHx of SCD	60/333 (18%)	5/20 (25%)	55/313 (17.6%)	0.402
ER on ECG	40/271 (14.8%)	4/19 (21.1%)	36/252 (14.3%)	0.423
QTc (ms)	421 (404-446)	423 (413-460)	421 (403-444)	0.445
TWI limb leads*	50 (13.7%)	5 (20.8%)	45 (13.2%)	0.293
fQRS duration (ms)	113 (105-121)	111.5 (106-119)	113 (105-121)	0.493
Duration of terminal 40 ms (ms)	34 (27-40)	37.5 (33-45)	33 (26-40)	0.13
RMS voltage of terminal 40 ms (μV)	31 (19-47)	25.5 (18-29)	31 (19-48)	0.121

Data presented as median (IQR) or numbers (percentages). * includes all limb leads. CMR: cardiac magnetic resonance, ETT: exercise treadmill test, FHx: family history, SCD: sudden cardiac death, ER: early repolarization pattern on ECG, QTc: corrected QT interval, TW: T-wave inversion, fQRS: filtered QRS, RMS: root mean square

Table 2: Characteristics of patients with mitral valve prolapse (MVP):

	MVP (n=34)	AMVP (n=24)	MVP+ (n=10)	P value
Age (years)	41 (33-49)	43.5 (35.5-49)	35 (26-49)	0.369
Gender (female)	22 (64.7%)	16 (66.7%)	6 (60%)	0.712
Ethnicity (white)	26 (76.5%)	18 (75%)	8 (80%)	0.754
Circumstance of death (exercise)	3/23 (13%)	3/17 (17.1%)	0/6 (0%)	0.27
Date of arrest (year)	2014 (2011-2016)	2014 (2011-2017)	2012 (2006-2014)	0.071
FHx of SCD	5/29 (17.2%)	5/20 (25%)	0/9 (0%)	0.099
Prolapse location (bileaflet)	19/33 (57.6%)	16/23 (69.6%)	3/10 (30%)	0.0346
MR severity (mild)	24/33 (72.7%)	16 (69.6%)	8 (80%)	0.536
LGE on CMR	4/19 (21.1%)	3/13 (23.1%)	1/6 (16.7%)	0.750
ER on ECG	5/29 (17.2%)	4/19 (21.1%)	1/10 (10%)	0.454
QTc (ms)	430 (414-462)	423 (412-460)	447 (420-462)	0.529
TWI inferior leads	24/33 (72.7%)	18/23 (78.3%)	6/10 (60%)	0.279
fQRS duration (ms)	113 (106-120)	111.5 (106-119)	118 (105-131)	0.321
Duration of terminal 40 ms (ms)	43 (36.5-46)	37.5 (33-45)	52.5 (42-79)	0.051
RMS voltage of terminal 40 ms (μV)	18 (16-26)	25.5 (18-29)	16 (11-23)	0.047
PVC on ETT	7/11 (63.6%)	4/7 (57.1%)	2/4 (50%)	0.819
PVC on monitoring	10/16 (62.5%)	5/10 (50%)	5/6 (83%)	0.182
Frequent PVCs on monitoring	4/7 (57.1%)	3/5 (60%)	1/2 (50%)	0.809

Data presented as median (IQR) or numbers (percentages). FHx: family history, SCD: sudden cardiac death, MR: mitral regurgitation, LGE late gadolinium enhancement, CMR: cardiac magnetic resonance, ER: early repolarization pattern, QTc: corrected QT interval, TWI: T-wave inversion, fQRS: filtered QRS, PVC: premature ventricular contraction, ETT: exercise treadmill test.

Table 3: detailed description of the evaluation and the final diagnosis of all patients with MVP:

ID	Demo.	Dx	Strength of Dx	CMR	ETT	Echo	Monitoring
AMVP							
267	51 M	IVF	Probable	No comment on LGE	Borderline. PVCs with exercise reported.	Bileaflet prolapse. +3 MR	NA
468	41 M	IVF	Definite	Mid-anterolateral wall LGE.	Normal.	Mild prolapse. +1 MR	NA
1626	57 F Arrest with Exercise	IVF	Definite	No LGE	PVCs increased with exercise. Single morphology: RBB-like, superior axis and transition in V3	Bileaflet prolapse with moderate to severe MR	2 Holters. Max. PVC burden: 7.4%, fastest NSVT: 294 b/min for 9 beats.
4003	33 M	IVF	Probable	No LGE	Reported PMVT arrest in recovery requiring shock. No chest pain.	Bileaflet prolapse. Mild prolapse with mild MR.	NA
4015	34 F Arrest with mild activity	IVF	Definite	No comment on LGE	Normal. No PVCs.	Mild prolapse with mild MR. Location not reported.	NA
4258	51 M	IVF	Possible	No LGE	NA	Severe MR.	NA
4380	47 M Arrest with exercise	IVF	Definite	No LGE	Multi-focal PVCs reported, including triplets.	Bileaflet prolapse. Mild prolapse with mild MR.	NA
4622	45 F	IVF	Definite	Small area of LV subendocardial fibrosis in basal and mid anterior wall. Normal segmental and global function.	Performed. No report available.	Anterior prolapse. Mild MR	NA
5288	45 F Arrest with mild activity	IVF	Probable	NA	NA	Bileaflet prolapse. Mild prolapse. Moderate MR	1 Holter. PVC burden: 5%, (multifocal PVCs) fastest NSVT 195 b/min for 4 neats.
5804	43 F Arrest at rest	IVF	Definite	No comment on LGE	No PVCs	Bileaflet prolapse. Severe MR.	1 Holter: PVC burden 1.1%. No NSVT.

6171	29 M Arrest at rest	IVF	Probable	No LGE	NA	Bileaflet prolapse. Moderate MR	NSVT on ICD
6173	51 M Arrest at rest	IVF	Possible	Transmural LGE of apical inferior wall.	Ischemic changes reported.	Posterior prolapse. Severe MR	NA
6327	45 M Arrest at rest	IVF	Possible	No comment on LGE	Normal. No PVCs	Posterior prolapse. Mild to moderate MR	NA
6362	32 F Arrest with mild activity	IVF	NA	Performed. No LGE	Performed. Reported to have PVCs with exercise (up-to couplets).	Bileaflet prolapse. Mild MR.	NA
6429	41 F Arrest at rest	IVF	Definite	NA	NA	Posterior prolapse. Mild to moderate MR	NA
6446	17 F	IVF	Definite	NA	NA	Bileaflet prolapse. Moderate MR	NA
7154	44 F Arrest with mild activity	IVF	Probable	No LGE	NA	Posterior prolapse. Mild MR	NA
7171	52 F Arrest with exercise	IVF	Probable	NA	Performed	Bileaflet prolapse. Mild MR	1 Holter: PVC burden:10%, fastest NSVT: 156 b/min for 4 beats
7248	41 F Arrest during an argument	IVF	Possible	No comment on LGE	Performed. Unifocal frequent PVCs during exercise from report. No strips	Bileaflet prolapse. Mild MR. Borderline prolapse	yes
7967	37 F Arrest at rest	IVF	Definite	No comment on LGE	NA	Bileaflet prolapse. Mild MR	NA
8124	41 F Arrest at rest	IVF	Definite	No LGE	PVCs in recovery, RBB-like, superior axis, V5 transition.	Bileaflet prolapse. Mild MR	1 Holter: PVC burden: <1%
8278	54 F Arrest with mild activity	IVF	Definite	No comment on LGE	NA	Bileaflet prolapse. Mild MR	NA
8777	46 F Arrest at rest	IVF	Definite	No LGE	Performed. Single PVCs, mainly in recovery, RBB-like, superior axis, V3 transition	Bileaflet prolapse. Mild MR	NA

8933	19 F	IVF	NA	No LGE	NA	Bileaflet prolapse. Mild MR	NA
MVP+							
181	32 F	DCM (LMNA)	Definite	NA	PVCs with exercise reported.	Borderline prolapse. Location and severity not reported	1 Holter: Reported PVCs and NSVT
1469	55M Arrest at rest	spasm	Probable	NA	NA	Posterior prolapse. Mild prolapse with +1 MR.	1 Holter: Reported PVCs
3050	35 F Arrest with mild activity	ARVC	Possible	No LGE.RV RWMA	Resting PVCs.	Anterior prolapse. Trivial MR. Borderline prolapse	1 Holter. PVC burden: 2% (2 close morphologies). No NSVT
4011	26 M	BrS	Definite	No comment on LGE	PVCs reported.	Anterior prolapse with mild MR. Borderline prolapse	NA
4265	55 M Arrest with mild activity	Sarcoid (Biopsy)	Definite	Mild RV dysfunction. No comment on LGE	Multi-focal PVCs reported.	Anterior prolapse. Mild to moderate MR. Borderline prolapse	NA
5344	40 F	LQTS	Probable	No comment on LGE	PVCs started in recovery, single morphology: RBB-like, inferior axis, V6 transition.	Posterior prolapse. Mild MR.	NA
5720	49 F Arrest at rest	LQTS	Definite	No LGE	No PVCs	Posterior prolapse. mild MR.	NA
5859	17 M	CPVT	Possible	No LGE	Performed. No available tracings.	Bileaflet prolapse. Trace MR	1 Holter. PVC burden:12.4%, 20 beats NSVT (rate: NA)
6166	25 F Arrest with mild activity	LQTS	Possible	No LGE	Single PVC at stage 4: RBB-like and inferior axis	Posterior prolapse. Mild prolapse with mild MR.	3 Holters. no PVCs
7355	Arrest with mild activity	UCM	Definite	Patchy subepicardial LGE in the mid inferolateral, inferior and anterolateral segments.	NA	Bileaflet prolapse. Mild MR	NA

AMVP: arrhythmic mitral valve prolapse (MVP+IVF), MVP+: MVP with an alternative diagnosis for cardiac arrest. Demo.: demographics, Dx: diagnosis, M:male, IVF: idiopathic ventricular fibrillation, NA: non-available, LGE: late gadolinium enhancement, PVC: premature ventricular contraction, MR: mitral regurgitation, RBBB: right bundle branch block, NSVT: non-sustain ventricular tachycardia, DCM: dilated cardiomyopathy, LMNA: Lamin, ARVC: arrhythmic right ventricular cardiomyopathy, BrS: Brugada syndrome, LQTS: Long QT syndrome, CPVT: Catecholaminergic polymorphic ventricular tachycardia, UCM: unclassified cardiomyopathy

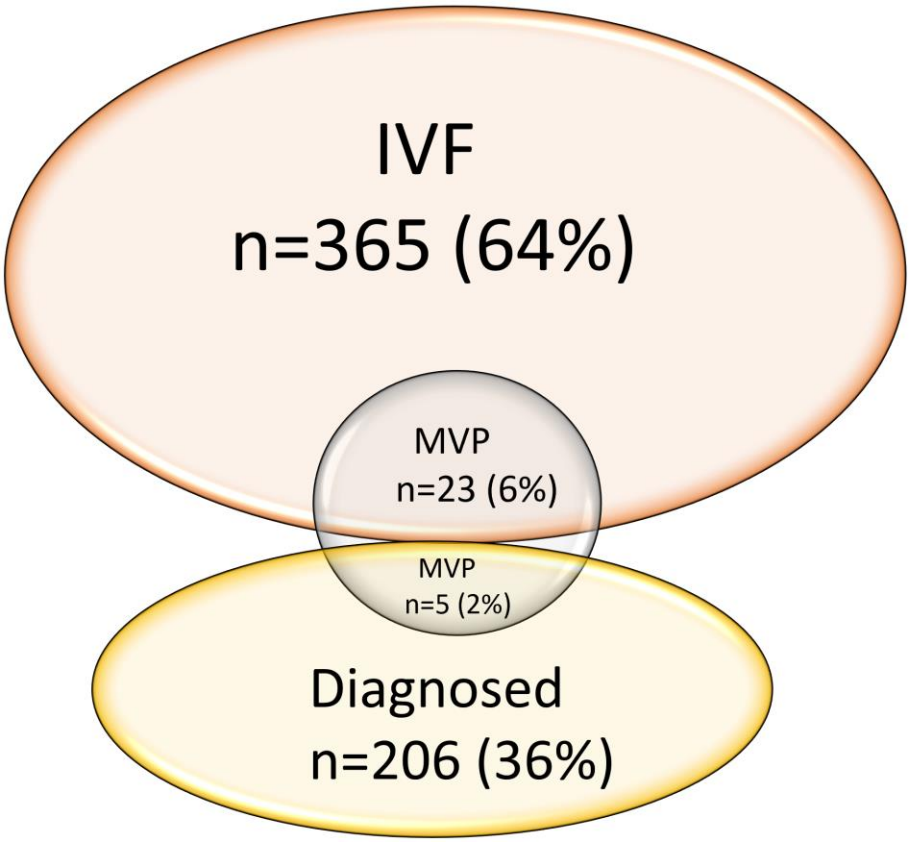


Figure1: The prevalence of mitral valve prolapse in patients with unexplained cardiac arrest (UCA). IVF: idiopathic ventricular fibrillation, MVP: mitral valve prolapse. * Only definite MVP were included.

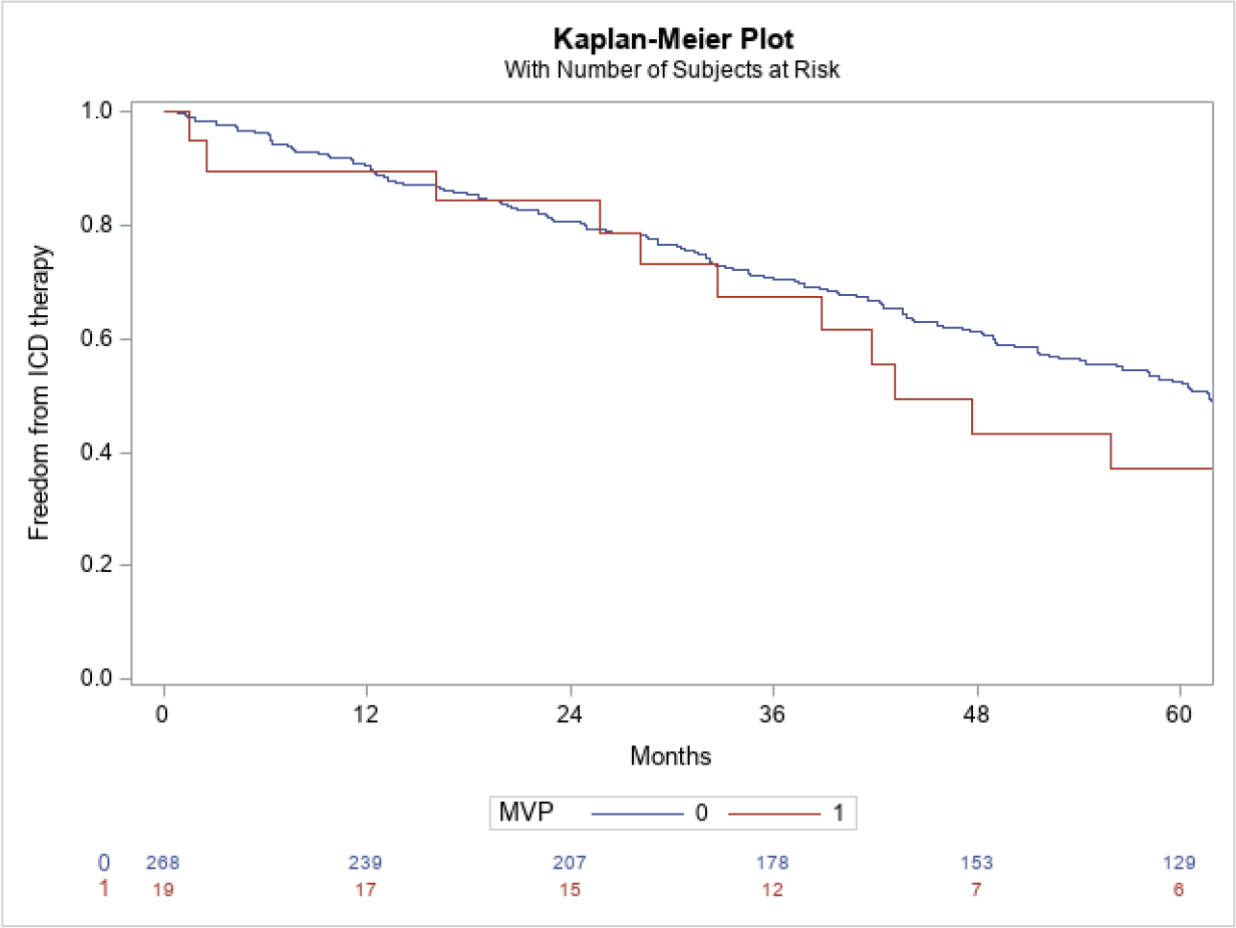


Figure 2: Survival free from appropriate ICD therapies for idiopathic ventricular fibrillation patients with and without mitral valve prolapse. MVP: mitral valve prolapse, ICD: implantable-cardioverter defibrillator.

Supplementary table 1: Pre-specified diagnostic criteria

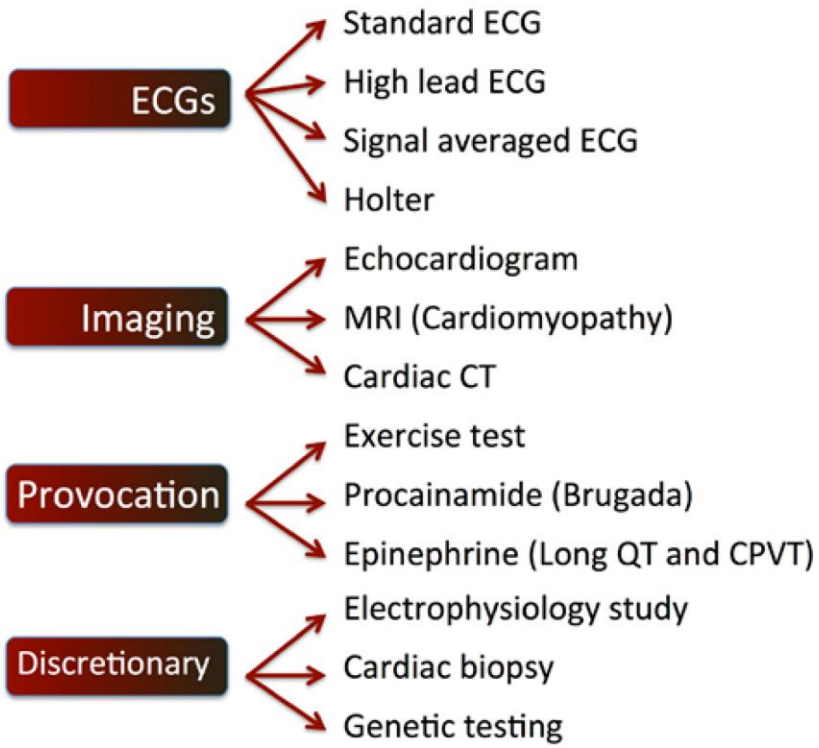
	Strength = Definite	**Strength = Probable**	**Strength = Possible**
LQTS	LQTS risk score≥3.5 in the absence of a secondary cause for QT prolongation *and/or* Unequivocally pathogenic variant in one of the LQTS genes *and/or* QTc≥500ms in repeated 12-lead electrocardiogram (ECG) in the absence of a secondary cause for QT prolongation	QTc between 480 and 499 ms in repeated 12-lead ECGs in a patient with unexplained syncope in the absence of a secondary cause for QT prolongation and in the absence of a pathogenic variant	LQTS risk score 2.0-3.5 in the presence of a family history of definite LQTS that is genotype negative or when genetic testing has not been performed
BrS	ST elevation with type 1 morphology ≥2mm in ≥1 of the right precordial leads V1-V2 positioned in the 4^{th}, 3^{rd} or 2^{nd} intercostal spaces, either spontaneously or after provocative drug test with IV class 1 drugs	Unequivocal pathogenic variant in *SCN5A* leading to decreased Nav1.5 function in presence of family history of definite BrS or in the context of a molecular autopsy	ST elevation with type 2 morphology and provocative testing has not been performed in presence of family history of definite BrS

CPVT	Structurally normal heart, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT or polymorphic ventricular premature beats (VPBs) or VT in an individual younger than 40 years *and/or* Presence of an unequivocal pathogenic variant *and/or* Family members of a CPVT index case with a normal heart who manifest exercise-induced premature ventricular contractions or bidirectional/polymorphic VT	Structurally normal heart, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT or polymorphic ventricular premature beats (VPBs) or VT in an individual older than 40 years	NA
HCM	Wall thickness ≥ 15mm (z-score ≥ 2 in children) in one or more LV myocardial segments that is not explained solely by loading conditions (e.g. SBP>160), excluding isolated basal septal hypertrophy in the elderly *and/or* Wall thickness ≥ 13 mm in 1st degree relatives of patients with definite HCM or with a pathogenic variant	NA	Wall thickness 13-14 mm in one or more LV myocardial segments that is not explained solely by loading conditions, (e.g. SBP>160), excluding isolated basal septal hypertrophy in the elderly, in the absence of 1st degree relatives of patients with definite HCM

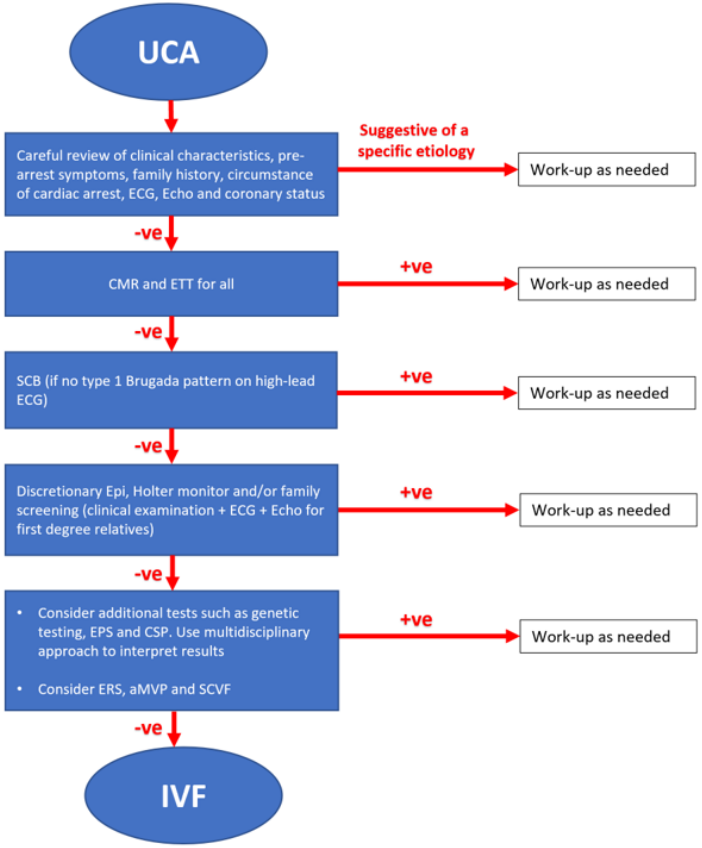
DCM	LV systolic dysfunction (LVEF<50%) AND enlargement, that is not explained by abnormal loading conditions, coronary artery disease, or a recent cardiac arrest	NA	NA
ARVC	Task Force criteria: 2 major or 1 major and 2 minor criteria or 4 minors from different categories	Task Force criteria: 1 major and 1 minor or 3 minor criteria from different categories	Task Force criteria: 1 major or 2 minor criteria from different categories.
LVNC	LVNC diagnosed by TTE or CMR	NA	NA
UCM	Unclassified cardiomyopathy: Presence of cardiomyopathy not fulfilling diagnostic criteria for the previous 4 other entities. E.g. Presence of significant fibrosis on magnetic resonance. Describe clinical findings in comments.	UCA/SCD with a pathogenic or likely pathogenic variant in a cardiomyopathy gene but no cardiomyopathy phenotype.	NA
Myocarditis	Endomyocardial biopsy-confirmed myocarditis (Dallas criteria)	Clinically-suspected myocarditis according to published criteria including CMR evidence, in the absence of an endomyocardial biopsy	Clinically-suspected myocarditis according to published criteria) in the absence of cardiac magnetic resonance imaging and endomyocardial biopsy

Coronary spasm	Evidence of angina in the absence of fixed coronary artery stenosis >50% AND Transient ischemic ECG changes during the spontaneous episodes and/or a positive acetylcholine/ergonovine test showing evidence of >90% coronary vasoconstriction (Appendix 5)	Polymorphic VT/VF in the absence of fixed coronary artery stenosis >50% or another etiology AND a positive acetylcholine/ergonovine test showing evidence of >90% coronary vasoconstriction	Evidence of nitrate-responsive angina in the absence of transient ischaemic ECG changes and coronary artery spasm

LQTS: Long QT syndrome, BrS: Brugada Syndrome, CPVT: Catecholaminergic polymorphic ventricular tachycardia, HCM; Hypertrophic cardiomyopathy, SBP: systolic blood pressure, LVEF: left ventricular ejection fraction, DCM: Dilated cardiomyopathy, ARVC: Arrhythmogenic right ventricular cardiomyopathy, LVNC: Left ventricular non-compaction, UCM: Unclassified cardiomyopathy, CMR: cardiac magnetic resonance imaging. Published in Davies B et al. The Hearts in Rhythm Organization: A Canadian National Cardiogenetics Network. CJC Open (2020).



Supplementary Figure 1: Diagnostic cascade for unexplained cardia arrest in the CASPER registry. ECG: electrocardiogram, MRI: magnetic resonance imaging, CPVT: catecholaminergic polymorphic ventricular tachycardia. Herman et al *Circ Arrhythm Electrophysiol*. 2016;9:e003619. DOI: 10.1161/CIRCEP.115.003619.)



Strength of diagnosis*:

Definite IVF	Probable IVF	Possible IVF
All high-yield tests were performed (CMR, ETT and SCB)	1 high-yield test was not performed (CMR, ETT or SCB)	>1 high-yield test were not performed (CMR, ETT or SCB)

Supplementary Figure 2: Proposed algorithm for idiopathic ventricular fibrillation diagnosis and criteria for strength of diagnosis. aMVP: arrhythmic mitral valve prolapse, CMR: cardiac MRI, challenge, CSP: coronary spasm provocation, ECG: electrocardiogram, Echo: echocardiogram, Epi: Epinephrine challenge test, EPS: electrophysiology study, ERS: early repolarization syndrome, ETT: exercise-treadmill test, IVF: idiopathic ventricular fibrillation, SCB: sodium channel blocker, SCVF: short-coupled ventricular fibrillation, UCA: apparently unexplained cardiac arrest

Baseline characteristics:

1. Age
2. Gender
3. BSA

Pre-specified echo features:

1. LA volume [1]
2. Left ventricular systolic diameter [1]
3. Left ventricular diastolic diameter [1]
4. Mitral regurgitation severity [2]
5. Location of prolapse (anterior vs posterior)
6. Maximum Leaflet thickness

   (the leading to the trailing edge of the thickest area of the midportion of the leaflet, excluding focal areas of thickness and chordae) [3]

7. Presence of myxomatous valve

   (defined as maximum leaflet thickness $\geq$ 5 mm) [3, 4]

8. Leaflet length (anterior)

(The maximum length obtained in diastole in the parasternal long axis view)[5, 6]

9. Hight of the prolapse

(The maximum excursion of the leaflet beyond the mitral annular plane as defined by a line connecting the inferolateral mitral annulus to the aortomitral junction during systole)[6]

10. Presence of redundancy

(Assessed in the parasternal short-axis view at the edges of the mitral valve and defined qualitatively as a disproportionate increase in the circumference of the leaflets relative to chamber size, so they had undulating appearance during valvular opening) [4]

11. Presence of flail leaflet [2]

12. Mitral annulus disjunction (MAD)

13. (Defined as a separation between the LA valve junction and the atrial aspect of the LV free wall in parasternal long axis view)[7]

14. MAD length

15. (Measured from the LA wall–posterior MV leaflet junction to the top of the LV infero-basal wall during end systole in parasternal long axis view)[8, 9]

16. Mitral annular diameter

17. (Measured from the junction between the posterior leaflet and the left atrium wall and the anterior leaflet and the aortic valve in the parasternal long axis view at end-diastole).[5]
18. Mitral annular calcification
19. (Graded by the how much of the circumference of the annulus being involved: <180, <270 or ≥ 270).[10]
20. Pickelhaube sign (high-velocity systolic signal TD)[11]
21. Tricuspid valve prolapse
22. (Defined as excessive billowing of tricuspid valve into the right atrium, associated with redundancy of tricuspid leaflet)[2]

Calculated parameters:

1- LA volume index
2- LV mass index
3- Number of prolapsed leaflets
4- MA diameter / anterior leaflet length ratio

References:

1. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;28:1-39 e14.

2. Zoghbi WA, Adams D, Bonow RO, et al. Recommendations for Noninvasive Evaluation of Native Valvular Regurgitation: A Report from the American Society of Echocardiography Developed in Collaboration with the Society for Cardiovascular Magnetic Resonance. *J Am Soc Echocardiogr.* 2017;30:303-371.

3. Freed LA, Levy D, Levine RA, et al. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;341:1-7.

4. Marks AR, Choong CY, Sanfilippo AJ, Ferre M, Weyman AE. Identification of high-risk and low-risk subgroups of patients with mitral-valve prolapse. *N Engl J Med.* 1989;320:1031-1036.

5. Nordblom P, Bech-Hanssen O. Reference values describing the normal mitral valve and the position of the papillary muscles. *Echocardiography.* 2007;24:665-672.

6. Han Y, Peters DC, Salton CJ, et al. Cardiovascular magnetic resonance characterization of mitral valve prolapse. *JACC Cardiovasc Imaging.* 2008;1:294-303.

7. Hutchins GM, Moore GW, Skoog DK. The association of floppy mitral valve with disjunction of the mitral annulus fibrosus. *N Engl J Med.* 1986;314:535-540.

8. Eriksson MJ, Bitkover CY, Omran AS, et al. Mitral annular disjunction in advanced myxomatous mitral valve disease: echocardiographic detection and surgical correction. *J Am Soc Echocardiogr.* 2005;18:1014-1022.

9. Carmo P, Andrade MJ, Aguiar C, Rodrigues R, Gouveia R, Silva JA. Mitral annular disjunction in myxomatous mitral valve disease: a relevant abnormality recognizable by transthoracic echocardiography. *Cardiovasc Ultrasound.* 2010;8:53.

10. Eleid MF, Foley TA, Said SM, Pislaru SV, Rihal CS. Severe Mitral Annular Calcification: Multimodality Imaging for Therapeutic Strategies and Interventions. *JACC Cardiovasc Imaging.* 2016;9:1318-1337.

11. Muthukumar L, Rahman F, Jan MF, et al. The Pickelhaube Sign: Novel Echocardiographic Risk Marker for Malignant Mitral Valve Prolapse Syndrome. *JACC Cardiovasc Imaging.* 2017;10:1078-1080.

CHAPTER 5: PROTOCOL OF A MATCHED CASE-CONTROL STUDY ENTITLED:

PREDICTINGRESUSCITATED CARDIAC ARREST WITH ECHOCARDIOGRAPHIC AND DOPPLER IMAGES IN CONTEMPORARILY TREATED MITRAL VALVE PROLAPSE (PREDICT-MVP)

The objective of the proposed study is to confirm the findings from the previous study (CHAPTER 4) in a larger sample of patients who did not have cardiac arrest. The proposed study also aims at better quantifying the association between pre-specified echocardiographic features and cardiac arrest.

Short Title: PREDICT-MVP

Authors: Wael Alqarawi [a,b], Girish Nair [b] , George Wells [b,d], Andrew D. Krahn [c], Ian Burwash [b]

This manuscript is under preparation. The protocol is to be registered at *clinicaltrials.gov* and published in a peer-review journal.

The student (WA) was first author, for which he was responsible for study design, manuscript preparation and revision. Drs. Wells, Nair, and Krahn provided guidance and feedback.

Title: Predicting Cardiac Arrest with Echocardiographic and Doppler Imaging in Contemporarily Treated Mitral Valve Prolapse (PREDICT-MVP): A Protocol for a Matched Case Control Study

Short Title: PREDICT-MVP

Authors: Wael Alqarawi [a,b], Girish Nair [b], George Wells [b,d], Andrew D. Krahn [c], Ian Burwash [b]

Affiliations:

a) School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada

b) Division of Cardiology, University of Ottawa Heart Institute, Ottawa, Canada

c) Center for Cardiovascular Innovation, Division of Cardiology, University of British Columbia, Canada

d) Cardiovascular Research Methods Centre, University of Ottawa Heart Institute, Canada

Word Count: 2885

Correspondence: Wael Alqarawi

University of Ottawa

40 Ruskin street,

Ottawa, ON, Canada.

K1Y 4W7

Phone: (613) 696 7402

E-mail: walqarawi@ottawaheart.ca

Conflict of interest : The authors have no conflict of interest to disclose.

Background:

Mitral valve prolapse (MVP) is seen in 1-3% of the general population.[1, 2] While generally regarded as a benign condition, it has been suggested that it is associated with sudden cardiac death (SCD).[3] Multiple studies have examined the prevalence of MVP in patients with SCD.[4-6] Sriram et al[5] reviewed 24 patients who survived SCD and found MVP in 10 of them (42%), which is substantially higher than the prevalence of MVP in the general population. Basso et al[4] reported a 7% prevalence of MVP in young adults with SCD where structural heart disease was excluded. More recently, Nalliah et al[2] performed a meta-analysis of studies examining the prevalence of MVP in unexplained SCD patients and found it to be 11.7%.

Limited studies have examined the annual risk of SCD in patients with MVP due to the low incidence. However, prospective studies have reported an annual risk of as high as 0.4% which is higher than the risk of SCD in the general population.[7-9] Moreover, Grigioni et al reported an annual risk of SCD of 1% in patients with MVP and a flail leaflet which is similar to the risk of SCD post myocardial infarction (MI).[10, 11]It is possible that only a small subset of patients with MVP are at a high risk of SCD just like patients post MI for example. Unlike post MI patients, however, there are no established predictors of SCD in MVP patients that allow us to identify these patients, rendering their management quite challenging. As such, there is a need to identify predictors of SCD that can inform decisions about effective primary prevention therapies such as implantable cardioverter-defibrillator (ICD) in these patients. Also, identifying this subset of high-risk patients will facilitate prospective studies to better estimate their risk of SCD and the effect of multiple proposed therapies such as valve repair and catheter ablation on mitigating the risk of SCD.[12-14]

Echocardiography is an excellent risk stratification tool as it is clinically indicated in the assessment of patients with MVP, easily available, non-invasive and relatively inexpensive. Echocardiographic features such as redundant mitral leaflet, flail leaflet and mitral regurgitation (MR) have been found to be associated with SCD in patients with MVP. [8, 10, 15] However, these associations were identified in relatively old studies where the management of conditions associated with MVP such as MR, left ventricular dilatation and dysfunction is quite different than current approaches, and can affect the risk of SCD. Indeed, more recent studies that re-examined some of these features have found conflicting results.[15, 16] Over the last decade, multiple promising echocardiographic predictors of SCD have been described such bileaflet involvement, mitral annular disjunction (MAD) and spiked systolic high-velocity signal on tissue Doppler of the lateral mitral annulus.[5, 17, 18] These reports are limited, however, by the lack of a control group and/or the use of surrogates of SCD such as ventricular arrhythmias on Holter monitoring or fibrosis on cardiac magnetic resonance (CMR) rather than SCD itself. In their highly cited study, Sriram et al[5] reported 10 patients with MVP who survived SCD and had no identifiable electrical or structural heart disease despite extensive work-up. They suggested that the cause of SCD in these patients is likely MVP. All these patients had bileaflet prolapse hence the term "Malignant Bileaflet Mitral Valve Prolapse Syndrome" was proposed to recognize this subgroup of patients with high risk of SCD. However, subsequent to this study, an international collaboration involving 9 tertiary centers reviewed 32 additional patients with MVP and unexplained SCD and reported a prevalence of bileaflet prolapse of 84%.[19] Moreover, only 70% of patients had bileaflet prolapse in the study by Basso et al[4] where autopsies of patients with isolated MVP as the only potential cause of SCD were examined. While it is widely believed that

bileaflet involvement is a predictor of SCD, these studies do not prove this association due to the lack of a control group of MVP patients without SCD and, more importantly, they do not provide a quantitative measure of the strength of association and the interaction with other predictors.

Similarly, interest has been reignited recently in the role of MAD related arrhythmogenesis in patients with MVP.[3, 17, 20] In the international collaborative study of patients with unexplained SCD and MVP, MAD was present in all patients that had echocardiographic images available for review.[19] Does that suggest that MAD is a predictor of SCD in patients with MVP? Perhaps, but without a control group, one can not be sure that MAD is not a common feature of MVP. Indeed, Hutchins et al[21] were the first to systematically examine myxomatous MVP for the presence of MAD. They examined 900 autopsies and reported a prevalence of (23/25) 92% of MAD in patients with myxomatous MVP. In addition, MAD, independent of MVP, was found to be associated with ventricular arrythmias and cardiac arrest.[20] As such, the role of MAD in predicting ventricular arrythmias or SCD in patients with MVP remains unclear.

Until there is a strong evidence of association for any of the proposed SCD predictors in patients with MVP, any effort to examine interventions aimed at reducing that risk are likely to face significant challenges and, arguably, are premature at this point of time. As such, our endeavour is to test the association between pre-specified echocardiographic features SCD in patients with MVP.

Study objective:

Test and quantify the association between pre-specified echocardiographic features in MVP and cardiac arrest.

Methods:

This will be a matched case control study of MVP patients with and without cardiac arrest. Case control design was chosen due to limited number of "cases" and matching was performed on variables confounding the relationship between echocardiographic features and cardiac arrest.

Cases:

Patients with MVP that have survived SCD will constitute cases. These patients will be identified from the CASPER registry (Cardiac Arrest Survivors with Preserved Ejection Fraction Registry)[22]. The details of the CASPER registry are available in the original publications.[22] In brief, patients that have survived cardiac arrest and were found to have a normal baseline electrocardiogram, no coronary artery disease, no left ventricular dysfunction and no apparent reversible cause were included. All patients in CASPER underwent extensive work-up to identify concealed causes of cardiac arrest. MVP patients will be identified by reviewing echocardiography reports of all patients enrolled in the registry. Baseline characteristics of these patients will be retrieved from CASPER and their echocardiographic images will be transferred to a secure database for independent review.

Controls:

Patients with MVP that have not had SCD will constitute the control group. The University of Ottawa Heart Institute (UOHI) echocardiography database (Xcelera) will be searched for codes assigned to MVP, and patients who had cardiac arrest will be identified by the presence of a defibrillator lead in the right ventricle (RV) will be excluded. Patients with concomitant structural disease will also be excluded. This step is to ensure that both cases and controls represent patients with isolated MVP with and without cardiac arrest, respectively. Note that *cases,* by definition, had no significant structural abnormalities, which was a pre-requisite for inclusion in CASPER. .

Controls will be selected by the following steps:

1- Codes for normal LV systolic function, normal RV size and systolic function, normal aortic root size, normal aortic valve structure and function, normal tricuspid valve and normal pulmonic valve will be combined with MVP codes to exclude patients with these abnormalities.
2- Duplicate studies (i.e. repeated echos for the same patient over time).The last available study will be included.
3- Before reviewing the pre-specified features, all echocardiography images will be screened by an experienced echocardiographer for any other rare significant abnormalities (e.g. severe pulmonary hypertension, congenital heart disease …ect), the presence of RV lead, poor images and incomplete images. These studies will also be excluded.

Matching:

Individualized matching on age (+/- 5 years), gender and body surface area (+/- 0.1 m^2) will be performed. These variables were chosen in view of their effect on the echocardiographic features being investigated.

Echocardiographic review:

Experienced echocardiographer(s) will review images. Pre-specified echocardiographic features will be recorded (list attached). These features were chosen based on previous literature or a biologic plausibility of an association with cardiac arrest. Definitions endorsed by guidelines or used in previous literature were utilized in the current study and are cited in the attached list.

Potential biases:

The major potential bias is "selection bias" in the *controls*. This is inherent to using a hospital echocardiography database. The concern is that patients who were referred for an echocardiography might not represent subjects with MVP in the general population. While this is a legitimate concern, it is unlikely to affect the result significantly for 4 reasons. First, our lab accepts referrals from general practitioners, who see a vast number of patients without known cardiac disease, and such subjects may better reflect the general population than subjects seen by cardiologists. Second, we have excluded patients with concomitant structural heart disease (i.e *cases* are either patients with a clinical presentation compatible with MVP or patients with incidental isolated MVP). Third, the reason to perform echocardiography in patients with MVP

is rarely due to malignant arrythmias (i.e. unlikely that patients with MVP with high risk for cardiac arrests will contaminate *controls*). Last, even if *controls* do not represent MVP in the general population, they do represent patients with isolated MVP seen by physicians and the results of this study will, at least, be generalizable to this group of patients, which is useful.

Statistical plan:

Summary of baseline characteristics will be described as median (interquartile) if continuous variable and counts (percent) if categorical. Proportions and ORs for each echocardiographic finding and pre-specified combinations will be presented. Pre-specified combinations were chosen based on clinical significance and include myxomatous valve with bileaflet involvement, myxomatous valve with MAD and bileaflet involvement with MAD. Continuous variables will be transformed to categorical variables based on established guidelines or will be divided into equal quartiles. P value ≤ 0.05 will be considered significant. Due to the hypothesized arrhythmogenicity of MAD itself and the fact that it might be present in a significant proportions of patients with each of the pre-specified echocardiographic features,[20] the independent association of each echocardiographic finding will be tested by adding it to MAD in a multivariate conditional logistic regression model. Inter- or intra-reader reliability will be assessed on 10% on the variables using Kappa statistics.

Assuming a prevalence of bileaflet involvement in controls of 33%,[23-25] a correlation coefficient of 0.5[19] and 25 cases of MVP with cardiac arrest who have available echocardiography images for review, 1:5 matching is required to provide 80% power to detect a statistically significant

association (odds ratio ≥ 5) with a type 1 error of 0.05. Bileaflet involvement was chosen given the availability of data in controls and its clinical importance (hence the proposed term "malignant bileaflet mitral valve prolapse syndrome").[5] Of note, the prevalence of MAD in patients with MVP in general is also reported to be 32% in a recent meta-analysis and, as such, this sample size will be sufficient to test the association between MAD and cardiac arrest as well.[26]

Discussion:

To our knowledge, our study will the first to provide evidence of association (or the lack of) between commonly cited echocardiographic features and cardiac arrest in patents with MVP. This is a critical initial step that will allow us to better define the subset of patients with MVP who are at high risk for cardiac arrest.

While it is widely believed that a small subset of MVP patients is at high risk for cardiac arrest, there is limited prospective studies that examined this cohort pf patients. This is likely due to the difficulty of performing such studies without identifying predicates of this small subset. One will need thousands of patients and many years of follow-up to study this rare event prospectively given the lack of any evidence-based risk stratification tool. We hope that our study will help identify easily applied predictors which will facilitate performing such prospective studies in the future.

Our study has multiple potential challenges. First, it does not consider clinical predictors and the interaction between these and echocardiographic features. Akin to the use of left ventricular ejection fraction post myocardial infarction to predict cardiac arrest, echocardiographic features can be used alone, and future studies can explore the role of clinical characteristics. Second, we have a small number of MVP patients who survived cardiac arrest. However, this will be the largest study to date and should have enough power to show strong associations (i.e. OR $\geq$ 5) as discussed above. It is possible that we could miss a weaker association although, practically speaking, a weaker association would be less useful clinically. Last, some of these patients might not have images with satisfactory quality to examine all the pre-specified features. However, at least some of commonly cited pre-specified features such as the number of prolapsed leaflets will likely be available for all patients which will be useful.

References:

1. Freed LA, Levy D, Levine RA, et al. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;341:1-7.

2. Nalliah CJ, Mahajan R, Elliott AD, et al. Mitral valve prolapse and sudden cardiac death: a systematic review and meta-analysis. *Heart.* 2019;105:144-151.

3. Miller MA, Dukkipati SR, Turagam M, Liao SL, Adams DH, Reddy VY. Arrhythmic Mitral Valve Prolapse: JACC Review Topic of the Week. *J Am Coll Cardiol.* 2018;72:2904-2914.

4. Basso C, Perazzolo Marra M, Rizzo S, et al. Arrhythmic Mitral Valve Prolapse and Sudden Cardiac Death. *Circulation.* 2015;132:556-566.

5. Sriram CS, Syed FF, Ferguson ME, et al. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. *J Am Coll Cardiol.* 2013;62:222-230.

6. Narayanan K, Uy-Evanado A, Teodorescu C, et al. Mitral valve prolapse and sudden cardiac arrest in the community. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2016;13:498-503.

7. Duren DR, Becker AE, Dunning AJ. Long-term follow-up of idiopathic mitral valve prolapse in 300 patients: a prospective study. *J Am Coll Cardiol.* 1988;11:42-47.

8. Nishimura RA, McGoon MD, Shub C, Miller FA, Jr., Ilstrup DM, Tajik AJ. Echocardiographically documented mitral-valve prolapse. Long-term follow-up of 237 patients. *N Engl J Med.* 1985;313:1305-1309.

9. Zuppiroli A, Rinaldi M, Kramer-Fox R, Favilli S, Roman MJ, Devereux RB. Natural history of mitral valve prolapse. *Am J Cardiol.* 1995;75:1028-1032.

10. Grigioni F, Enriquez-Sarano M, Ling LH, et al. Sudden death in mitral regurgitation due to flail leaflet. *J Am Coll Cardiol.* 1999;34:2078-2085.

11. Adabag AS, Therneau TM, Gersh BJ, Weston SA, Roger VL. Sudden death after myocardial infarction. *JAMA.* 2008;300:2022-2029.

12. Vaidya VR, DeSimone CV, Damle N, et al. Reduction in malignant ventricular arrhythmia and appropriate shocks following surgical correction of bileaflet mitral valve prolapse. *J Interv Card Electrophysiol.* 2016;46:137-143.

13. Alqarawi W, Birnie DH, Burwash IG. Mitral valve repair results in suppression of ventricular arrhythmias and normalization of repolarization abnormalities in mitral valve prolapse. *HeartRhythm Case Rep.* 2018;4:191-194.

14. Abbadi DR, Purbey R, Poornima IG. Mitral valve repair is an effective treatment for ventricular arrhythmias in mitral valve prolapse syndrome. *Int J Cardiol.* 2014;177:e16-18.

15. Dollar AL, Roberts WC. Morphologic comparison of patients with mitral valve prolapse who died suddenly with patients who died from severe valvular dysfunction or other conditions. *J Am Coll Cardiol.* 1991;17:921-931.

16. Turker Y, Ozaydin M, Acar G, et al. Predictors of ventricular arrhythmias in patients with mitral valve prolapse. *Int J Cardiovasc Imaging.* 2010;26:139-145.

17. Perazzolo Marra M, Basso C, De Lazzari M, et al. Morphofunctional Abnormalities of Mitral Annulus and Arrhythmic Mitral Valve Prolapse. *Circ Cardiovasc Imaging.* 2016;9:e005030.

18. Muthukumar L, Rahman F, Jan MF, et al. The Pickelhaube Sign: Novel Echocardiographic Risk Marker for Malignant Mitral Valve Prolapse Syndrome. *JACC Cardiovasc Imaging.* 2017;10:1078-1080.

19. Hourdain J, Clavel MA, Deharo JC, et al. Common Phenotype in Patients With Mitral Valve Prolapse Who Experienced Sudden Cardiac Death. *Circulation.* 2018;138:1067-1069.

20. Dejgaard LA, Skjolsvik ET, Lie OH, et al. The Mitral Annulus Disjunction Arrhythmic Syndrome. *J Am Coll Cardiol.* 2018;72:1600-1609.

21. Hutchins GM, Moore GW, Skoog DK. The association of floppy mitral valve with disjunction of the mitral annulus fibrosus. *N Engl J Med.* 1986;314:535-540.

22. Krahn AD, Healey JS, Chauhan V, et al. Systematic assessment of patients with unexplained cardiac arrest: Cardiac Arrest Survivors With Preserved Ejection Fraction Registry (CASPER). *Circulation.* 2009;120:278-285.

23. Yeo TC, Lim MC, Cheng KL, See Tho ML, Ng WL, Choo MH. Clinical and echocardiographic features of mitral valve prolapse patients in a local population. *Singapore Med J.* 1996;37:143-146.

24. Seeburger J, Borger MA, Doll N, et al. Comparison of outcomes of minimally invasive mitral valve surgery for posterior, anterior and bileaflet prolapse. *Eur J Cardiothorac Surg.* 2009;36:532-538.

25. Delabays A, Jeanrenaud X, Chassot PG, Von Segesser LK, Kappenberger L. Localization and quantification of mitral valve prolapse using three-dimensional echocardiography. *Eur J Echocardiogr.* 2004;5:422-429.

26. Bennett S, Thamman R, Griffiths T, et al. Mitral annular disjunction: A systematic review of the literature. *Echocardiography.* 2019;36:1549-1558.

Supplementary materials:

Baseline characteristics:

4. Age
5. Gender
6. BSA

Pre-specified echo features:

23. LA volume [1]

24. Left ventricular systolic diameter [1]

25. Left ventricular diastolic diameter [1]

26. Mitral regurgitation severity [2]

27. Location of prolapse (anterior vs posterior)

28. Maximum Leaflet thickness

(the leading to the trailing edge of the thickest area of the midportion of the leaflet, excluding focal areas of thickness and chordae) [3]

29. Presence of myxomatous valve

(defined as maximum leaflet thickness $\geq$ 5 mm) [3, 4]

30. Leaflet length (anterior)

(The maximum length obtained in diastole in the parasternal long axis view)[5, 6]

31. Hight of the prolapse

(The maximum excursion of the leaflet beyond the mitral annular plane as defined by a line connecting the inferolateral mitral annulus to the aortomitral junction during systole)[6]

32. Presence of redundancy

(Assessed in the parasternal short-axis view at the edges of the mitral valve and defined qualitatively as a disproportionate increase in the circumference of the leaflets relative to chamber size, so they had undulating appearance during valvular opening) [4]

33. Presence of flail leaflet [2]
34. Mitral annulus disjunction (MAD)
35. (Defined as a separation between the LA valve junction and the atrial aspect of the LV free wall in parasternal long axis view)[7]
36. MAD length
37. (Measured from the LA wall–posterior MV leaflet junction to the top of the LV infero-basal wall during end systole in parasternal long axis view)[8, 9]
38. Mitral annular diameter
39. (Measured from the junction between the posterior leaflet and the left atrium wall and the anterior leaflet and the aortic valve in the parasternal long axis view at end-diastole).[5]
40. Mitral annular calcification
41. (Graded by the how much of the circumference of the annulus being involved: <180, <270 or $\geq$ 270).[10]
42. Pickelhaube sign (high-velocity systolic signal TD)[11]
43. Tricuspid valve prolapse
44. (Defined as excessive billowing of tricuspid valve into the right atrium, associated with redundancy of tricuspid leaflet)[2]

<u>Calculated parameters:</u>

5- LA volume index
6- LV mass index

7- Number of prolapsed leaflets

8- MA diameter / anterior leaflet length ratio

References

1. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2015;28:1-39 e14.

2. Zoghbi WA, Adams D, Bonow RO, et al. Recommendations for Noninvasive Evaluation of Native Valvular Regurgitation: A Report from the American Society of Echocardiography Developed in Collaboration with the Society for Cardiovascular Magnetic Resonance. *J Am Soc Echocardiogr.* 2017;30:303-371.

3. Freed LA, Levy D, Levine RA, et al. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;341:1-7.

4. Marks AR, Choong CY, Sanfilippo AJ, Ferre M, Weyman AE. Identification of high-risk and low-risk subgroups of patients with mitral-valve prolapse. *N Engl J Med.* 1989;320:1031-1036.

5. Nordblom P, Bech-Hanssen O. Reference values describing the normal mitral valve and the position of the papillary muscles. *Echocardiography.* 2007;24:665-672.

6. Han Y, Peters DC, Salton CJ, et al. Cardiovascular magnetic resonance characterization of mitral valve prolapse. *JACC Cardiovasc Imaging.* 2008;1:294-303.

7. Hutchins GM, Moore GW, Skoog DK. The association of floppy mitral valve with disjunction of the mitral annulus fibrosus. *N Engl J Med.* 1986;314:535-540.

8. Eriksson MJ, Bitkover CY, Omran AS, et al. Mitral annular disjunction in advanced myxomatous mitral valve disease: echocardiographic detection and surgical correction. *J Am Soc Echocardiogr.* 2005;18:1014-1022.

9. Carmo P, Andrade MJ, Aguiar C, Rodrigues R, Gouveia R, Silva JA. Mitral annular disjunction in myxomatous mitral valve disease: a relevant abnormality recognizable by transthoracic echocardiography. *Cardiovasc Ultrasound.* 2010;8:53.

10. Eleid MF, Foley TA, Said SM, Pislaru SV, Rihal CS. Severe Mitral Annular Calcification: Multimodality Imaging for Therapeutic Strategies and Interventions. *JACC Cardiovasc Imaging.* 2016;9:1318-1337.

11. Muthukumar L, Rahman F, Jan MF, et al. The Pickelhaube Sign: Novel Echocardiographic Risk Marker for Malignant Mitral Valve Prolapse Syndrome. *JACC Cardiovasc Imaging.* 2017;10:1078-1080.

CHAPTER 6: DISCUSSION

In this thesis, we proposed a standardized definition for IVF and used it to perform sensitivity analyses to test the association between MVP and IVF that we found in a large cohort of patients with UCA. Moreover, we identified features that appear to be more prevalent in AMVP patients, as compared to other MVP patients.

Multiple studies and case reports have suggested that MVP is an important cause of IVF, however, they suffered from the lack of a standardized definition of IVF and, as such, the potential of missing alternative diagnoses.[1, 2] This is important as the prevalence of MVP in these studies varies depending on the rigors to which alternative causes have been excluded.[1, 3, 4] The prevalence of MVP has been reported to be 2% when no alternative causes were excluded, 7% when structural, but not electrical causes were excluded, and 41% if all alternative causes were excluded.[1, 3, 4] We performed multiple sensitivity analyses based on our proposed definition of IVF to ensure that the prevalence is consistent regardless of the extent of evaluation performed in our cohort (i.e. unlikely that MVP is an innocent bystander). The other strength of our analysis is that it included patients from a national registry, with less risk of referral bias. This likely explains the different prevalence of MVP in patients with IVF in our study, as compared with the study by Sriram et al[1], which was the only other study that examined this question.

Identifying MVP patients at risk of cardiac arrest is important to institute preventative therapies. Previous studies have described phenotype features to identify this group of patients, however,

did not take into account the prevalence of these features in the patients with MVP who are not at elevated risk for cardia arrest.[1, 5] For example, while female gender is shown to be predominant in patients with MVP and SCD, this might simply be due to the fact that MVP is more common in females than males. Indeed, in the study by Freed et al[6], 3491 subjects (52% females) from the Framingham Heart Study were examined for the presence of MVP. They found that MVP was more common in females than males. Consequently, 60% of MVP patients were females, which is just slightly higher than the prevalence of females in case series of MVP with IVF.[5, 7] This emphasizes the importance of having a control group with MVP but no cardiac arrest or MVP with an alternative cause for cardiac arrest (i.e. MVP is likely an innocent bystander) to identify features that can distinguish patients with high risk of cardiac arrest [i.e. arrhythmic MVP (AMVP)].

Given the availability of echocardiogram (Echo) for all patients with MVP, it provides an excellent tool that can be used to distinguish AMVP from non-arrhythmic MVP. We found that bileaflet prolapse can distinguish AMVP patients from the general MVP population by comparing MVP patients with IVF to MVP with an alternative diagnosis in the CASPER registry. We plan to perform a matched case-control study with enough power to identify other features and to quantify the association between these features and AMVP. The hope is to find easily measured Echo features that are associated with AMVP, which will facilitate clinical care and research. Currently, for example, it is unclear who from the 1-2% in the general population with MVP need to be evaluated for the risk of SCD. By identifying features that distinguish AMVP patients, we can begin to select patients who are unlikely to benefit from any evaluation for the risk of SCD. This is imperative as questioning the risk of SCD for all MVP patients will

likely result in introducing unnecessary anxiety to patients and will overwhelm the heath care system with many patients with MVP who are at low risk for SCD. While the specificity of the features identified by our proposed study are not going to be tested, the results will inform future research to better select patients who are at higher risk of SCD in order to minimize the number of patients and years of follow-up required to prospectively assess that risk.

Once AMVP patients are better defined, future studies can then focus on quantifying the risk of SCD and examining the benefit of various therapeutic tools to mitigate that risk. We plan to conduct PREDICT-MVP-1 study (the protocol is included in CHAPTER 5) followed by PREDICT-MVP-2 study (Predicting Sudden Cardiac Death in Contemporarily Treated Mitral Valve Prolapse: A Retrospective Cohort Study), which will utilize data from the University of Ottawa Heart Institute (UOHI) echocardiography database, linked with provincial administrative health data at ICES and vital statistics database to determine the incidence of SCD in MVP patients, stratified by the presence of risk factors identified in PREDICT-MVP-1. These 2 studies will hopefully help better define AMVP patients and their risk of SCD. Once that's done, one can examine the effect of multiple primary prevention therapies on AMVP patients such as β-blockers, catheter ablation, mitral valve repair and ICD to better inform decisions about their management.[8-12]

References:

1. Sriram CS, Syed FF, Ferguson ME, et al. Malignant bileaflet mitral valve prolapse syndrome in patients with otherwise idiopathic out-of-hospital cardiac arrest. *J Am Coll Cardiol.* 2013;62:222-230.

2. Nalliah CJ, Mahajan R, Elliott AD, et al. Mitral valve prolapse and sudden cardiac death: a systematic review and meta-analysis. *Heart.* 2019;105:144-151.

3. Basso C, Perazzolo Marra M, Rizzo S, et al. Arrhythmic Mitral Valve Prolapse and Sudden Cardiac Death. *Circulation.* 2015;132:556-566.

4. Narayanan K, Uy-Evanado A, Teodorescu C, et al. Mitral valve prolapse and sudden cardiac arrest in the community. *Heart rhythm : the official journal of the Heart Rhythm Society.* 2016;13:498-503.

5. Han HC, Ha FJ, Teh AW, et al. Mitral Valve Prolapse and Sudden Cardiac Death: A Systematic Review. *J Am Heart Assoc.* 2018;7:e010584.

6. Freed LA, Levy D, Levine RA, et al. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med.* 1999;341:1-7.

7. Hourdain J, Clavel MA, Deharo JC, et al. Common Phenotype in Patients With Mitral Valve Prolapse Who Experienced Sudden Cardiac Death. *Circulation.* 2018;138:1067-1069.

8. Abbadi DR, Purbey R, Poornima IG. Mitral valve repair is an effective treatment for ventricular arrhythmias in mitral valve prolapse syndrome. *Int J Cardiol.* 2014;177:e16-18.

9. Alqarawi W, Birnie DH, Burwash IG. Mitral valve repair results in suppression of ventricular arrhythmias and normalization of repolarization abnormalities in mitral valve prolapse. *HeartRhythm Case Rep.* 2018;4:191-194.

10. Hosseini S, Rezaei Y, Samiei N, et al. Effects of mitral valve repair on ventricular arrhythmia in patients with mitral valve prolapse syndrome: A report of two cases. *Int J Cardiol.* 2016;222:603-605.

11. Syed FF, Ackerman MJ, McLeod CJ, et al. Sites of Successful Ventricular Fibrillation Ablation in Bileaflet Mitral Valve Prolapse Syndrome. *Circulation. Arrhythmia and electrophysiology.* 2016;9.

12. Wilde AA, Duren DR, Hauer RN, et al. Mitral valve prolapse and ventricular arrhythmias: observations in a patient with a 20-year history. *J Cardiovasc Electrophysiol.* 1997;8:307-316.