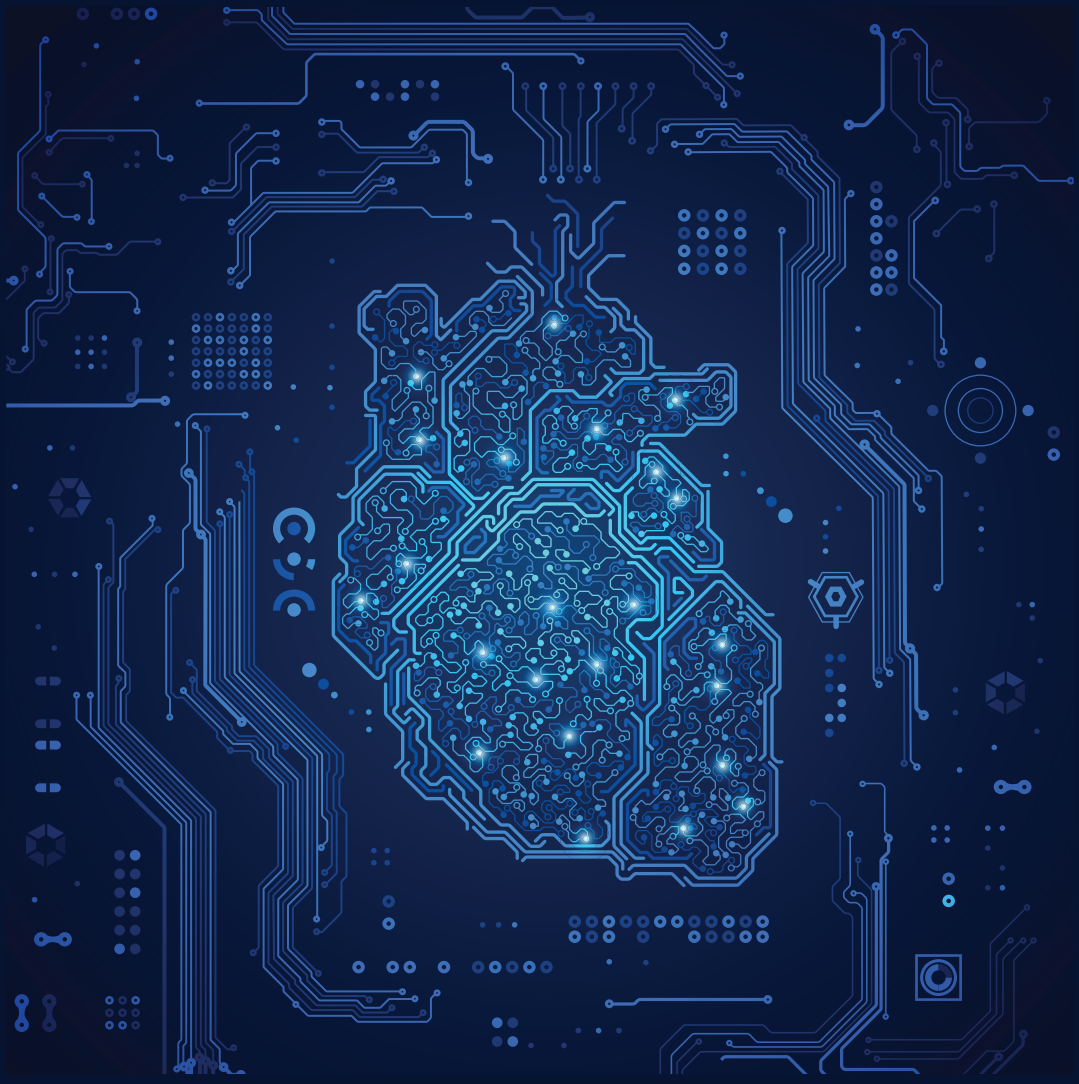
RISK STRATIFICATION IN ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

AN INTEGRATIVE APPROACH TOWARDS GENE-SPECIFIC INSIGHTS



Alessio Gasperetti

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Risk Stratification In Arrhythmogenic Right Ventricular Cardiomyopathy

An Integrative Approach Towards Gene-Specific Insights

Risico Stratificatie Bij Aritmogene Rechter Ventrikel Cardiomyopathie
Een Integrale Benadering Gebaseerd Op Gen-Specifieke Inzichten
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

donderdag 8 juni 2023 des middags te 2.15 uur

door

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geboren op 21 november 1993
te Milan, Italië

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“All that is gold does not glitter,
Not all those who wander are lost
The old that is strong does not wither,
Deep roots are not reached by the frost”

J.R.R. Tolkien

“There are only three kind of Lies:
Lies, damned Lies, and Statistics”

Marc Twain

“Prediction is very difficult,
especially if it's about the future!”

Niels Bohr

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PART I: Validation of the ARVC Risk Calculator

PART II: Refinement of the ARVC Risk Calculator

CHAPTER 1

PREFACE

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an hereditable heart muscle disease, with an estimated prevalence ranging between 1:1000 and 1:5000 individuals, although significant geographical differences exist[1]. The histological hallmark of this disease is the presence of myocardial fibro-fatty replacement at a ventricular level. While originally thought to be a disease involving exclusively the right ventricle (hence the name), recent studies have shown that the spectrum of disease phenotypes associated with ARVC comprises also forms that may be biventricular or involve the left ventricle in the early stages of disease. Patients with ARVC are at an increased risk of ventricular arrhythmias (VAs), with sudden cardiac death (SCD) episodes representing the most devastating events. These arrhythmic events are unfortunately more frequent in young, athletic, and apparently healthy individuals and pose a significant threat in terms of morbidity and mortality for affected patients[2].

While we hope that the truth of this statement may change in the future, to this day no causative therapy for ARVC is available and much of disease management is aimed at arrhythmic burden reduction and prevention of SCD events. The placement of implantable cardioverter defibrillators (ICDs) currently represents the cornerstone for sudden cardiac death prevention in patients with ARVC[3,4]. The placement of an ICD, however, is an invasive and expensive procedure that may cause significant psychological distress in young recipients. ICDs are also potentially associated with long-term lead complications, the risk of inappropriate shocks, and device infections, which are of particular relevance in a young population that may require this device for their entire lifetime[5]. The risk-benefit balance of placing an ICD should therefore be carefully weighted, especially in those patients diagnosed with ARVC but without any previous arrhythmic events at the time of disease diagnosis (often called “primary prevention” ARVC patients). The recommendation of ICD placement in these patients is particularly challenging. Exceedingly aggressive ICD implantation strategies have been critiqued, due to reports showing that over two-thirds of ICDs implanted in primary prevention ARVC patients never deliver a therapy, bringing only their complications to the table[6,7]. At the same time, a too lenient approach to ICD implantation may leave patients unprotected from potentially lethal arrhythmic events. For these reasons, over 20 years of scientific efforts have been dedicated to the difficult task of assessing the arrhythmic determinants of ARVC, in a complex quest towards individual patient arrhythmic risk estimation.

Thanks to great efforts of the international community, this process made a significant step forward in 2019, when a risk calculator for arrhythmic risk of ARVC patients was developed by a large international collaboration of centers from European and North American countries[8,9]. This risk

calculator weighted 7 clinical variables routinely collected in patients with ARVC (age, sex, RVEF, number of T wave inversion in a 12 lead ECG, 24-h premature ventricular contraction (PVC) burden estimated by an Holter-ECG, presence of non-sustained VT, and presence of recent cardiac syncope events) allowing for estimation of a patient's individual risk of developing an incident sustained ventricular arrhythmia over the upcoming 5 years of follow up. This calculator was made available online at www.arvcrisk.com.

The novel ARVC risk calculator was shown to outperform the risk stratification algorithm available at that time and quickly became an integrated part of clinical management of patients with ARVC in many cardiomyopathy units across the globe. With no external validation studies available and with most involved centers in its development being tertiary electrophysiology centers, however, some questions regarding its generalizability were raised. Additionally, since its development, areas of potential improvements and refinement (i.e. integration of the role of physical exercise or of programmed ventricular stimulation) were identified[10].

In this thesis, I leverage extensive multi-national research collaborations involving more than 20 centers in 8 countries to address those topics. **Chapter 2** provides a summary of the ARVC literature with a specific focus on risk stratification and the ARVC risk calculator. A historical perspective, as well as all the research steps behind the development of the current ARVC risk calculator are reported, to help the reader get accustomed with the main topic of discussion. As reflected in the title, this thesis is then divided in two main parts. The first part reports the external assessment and validation efforts of the originally published 2019 ARVC risk calculator. The second part then reports on additional studies that have been performed to further refine the performance of the risk calculator, with the future perspective of a gene-specific approach clearly in mind.

PART 1 – Validation of the original ARVC risk calculator tool

Chapter 3 and **Chapter 4** represent the first external studies addressing risk calculator performance. **Chapter 3** describes the first world-wide assessment of the performance of the ARVC risk calculator in an external cohort of patients from Italy fulfilling a definite diagnosis of ARVC. This chapter provides insights on the reliability of this risk stratification tool, describing a good concordance between observed and predicted arrhythmic events in this cohort. It also confirms the algorithm is better at identifying patients in need of an ICD than the currently available risk stratification consensus. This chapter, however, also identifies some of the limitations of the risk calculator, by

showing an underprediction of arrhythmic risk in patients with non-classical ARVC phenotypes such as those with a biventricular or left-dominant involvement.

Physical exercise and endurance training are well-known risk modifiers in patients with ARVC, with the risk of SCD in patients exercising increasing up to four-fold. Since its first publication, the reliability of the ARVC risk calculator in patients with an extensive personal history of physical exercise performance has been questioned. **Chapter 4** tackles this topic, showing a good performance of the model in a population of high-end competitive athletes diagnosed with ARVC. This study postulates that the additional risk associated with exercise may have already been captured within the variations of the clinical parameters weighted in the risk calculator and no correction for exercise is needed. **Chapter 5** closes the validation section of this thesis, summarizing more than two years of extensive networking and international collaboration efforts aimed at gathering a large validation cohort for an adequately powered external validation of the ARVC risk calculator. This study confirmed the good performance of the ARVC risk calculator in a large external validation cohort.

PART 2 – Improving the original ARVC risk calculator tool

While the efforts reported in **Chapters 3 to 5** clearly established the reliability of the ARVC risk calculator, many additional questions were still left unanswered.

Chapter 6 tackles the controversial topic of programmed ventricular stimulation (PVS) for risk stratification in primary prevention patients with ARVC. Prior studies have on this topic reported conflicting results: all of these studies were hampered by small patient sample sizes and by the inclusion of both patients with and without previous sustained VA events at the time of PVS performance. This study leveraged the largest cohort to date of primary prevention ARVC patients undergoing PVS (n=288 from 5 countries) to address this topic, showing a clear association between PVS results and the 5-year arrhythmic risk. Furthermore, a Bayesian analysis was performed to integrate PVS study results into the original risk calculator. An approach combining PVS results and the use of the ARVC risk calculator was shown to be superior to either of the individual components in predicting arrhythmic risk for these patients. The findings of this study are the basis upon which the current updated version of the online ARVC risk calculator website is based (www.arvcrisk.com).

The ARVC risk calculator was originally meant as a risk stratification tool to guide ICD implantation to be used at the time of first clinical assessment after disease diagnosis. Many ARVC patients, however, are followed up by dedicated providers for years, with many additional prognostic evaluations becoming available over time. A longitudinal risk stratification strategy to dynamically predict the risk of ventricular arrhythmias over time was lacking. **Chapter 7** and **Chapter 8** of this thesis address this topic. In **Chapter 7,** the prognostic role of multiple 24-h ECG Holter recordings is reported, with a particular focus on the value of sudden changes in the 24-h PVC burden (the so called "PVC spikes"). This chapter provides strong data supporting a longitudinal risk stratification strategy based on multiple Holter ECGs performed during follow up. **Chapter 8** further builds on Chapter 7, showing how a longitudinal assessment with the updating of any available clinical test into the risk calculator estimate is a reliable and effective strategy for longitudinal assessment of the arrhythmic risk of patients with ARVC.

Over the last few years, there has been strong interest within the ARVC community in risk stratification in patients with ARVC harboring a desmoplakin (*DSP*) genetic variant. *DSP* pathogenic variants are known to be associated with a biventricular / left-dominant ARVC phenotype, for which the current ARVC risk stratification tool may be inappropriate. The final **Chapter 9** reports the long-term outcomes of the largest *DSP*-ARVC cohort to ever be gathered in ARVC research, showing this disease to be extremely aggressive from an arrhythmic standpoint. It also builds on the findings of Chapter 2, addressing the performance of the ARVC risk calculator in a large cohort of patients harboring a *DSP* pathogenic variant who fulfill the criteria for risk calculator use (i.e. diagnosis per 2010 Task Force Criteria and no history of a sustained ventricular arrhythmia at diagnosis). This chapter shows underprediction of arrhythmic events in this population and marginal calibration of the risk calculator particularly in patients with left ventricular disease. These results strongly suggest the need for a future genotype-based risk stratification approach, which is also discussed in this chapter.

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CHAPTER 2

ARRHYTHMIC RISK STRATIFICATION IN ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY: REVIEW OF CURRENT EVIDENCE AND A MODERN PRACTICAL APPROACH

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Submitted

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiomyopathy characterized by a predominantly arrhythmic presentation and with the histological hallmark of scarring and/or fibro-fatty infiltration of the ventricular myocardium. Although in its first descriptions believed to be disease involving exclusively the right-ventricle (RV) (1), over the years biventricular and left dominant phenotypes have been increasingly described (2–4).

Numerous different underlying genetic variants can all lead to the development of ARVC. Although a significant variability in the association between genotype and phenotype is present (Table1), all forms of ARVC are inherently associated with an increased risk of sustained ventricular arrhythmias (VA) and sudden cardiac death (SCD). While ARVC is estimated to be almost 10 times less prevalent than hypertrophic cardiomyopathy, ARVC accounts for around 4% of all SCD at autopsy studies, around 10% of unexplained sudden cardiac arrests, and it is one of the most common causes of SCD among athletes (2,5,6). It is therefore crucial for clinicians treating patients with ARVC to be able to accurately assess the risk of VA/SCD of this condition, especially when interacting with patients without previous VA events (the so called “primary prevention” ARVC patients). The purpose of this review article is to summarize the large body of evidence that allowed the development of a modern tool for risk stratification in patients with ARVC and the best way to implement its use in the clinical workflow.

PATIENT MANAGEMENT AND ARRHYTHMIC RISK STRATIFICATION

To this day, unfortunately, no disease-modifying treatment is available for the treatment of ARVC. Therefore, the main focus of current consensus statements for ARVC is the reduction of the risk of SCD (7–9). Apart from risk factor reduction (i.e. physical exercise restriction), the cornerstone of SCD prevention in patients with ARVC is the placement of an implantable cardioverter defibrillator (ICD). In a young and active population with a potentially very long life-expectancy such

as the one that is often affected by ARVC, however, the potential absolute risk of SCD reduction achieved with ICDs should be carefully weighed against the risk of device-related complications. Multiple studies have in fact shown how both transvenous and subcutaneous ICDs are associated with complication and adverse events in these patients (10–12), with a meta-analysis showing a potential 3.9% pooled risk annual rate of inappropriate shocks and a 4.2% annual rate of other complications such as infection or lead malfunction for young patients implanted with ICD for the management of familial cardiomyopathies (13). Performing an accurate risk-benefit analysis of ICD implantations in patients with ARVC is therefore a critical part of the integrative management of these patients.

Known Predictors and Current Guidelines

Over the years, several studies have identified the associations between multiple demographic, clinical, genetic, and instrumental findings and the development of sustained VAs in patients with ARVC (**Table 2**). Among demographic characteristics, young age (with a particular peak of risk at in early adulthood) as well as male sex are well known predictors of VAs in this patient population (14–18), potentially due an effect caused by testosterone and other sex hormones (19,20). Numerous clinical red flags associated with an increased arrhythmic risk profile of patients with ARVC can be detected by non-invasive examinations, such as 12-lead ECGs (i.e. number of T wave inversions, QRS complex fractionation), 24-h Holters (i.e. premature ventricular contraction (PVC) daily burden, PVC spikes, non-sustained ventricular tachycardia (NSVT)), or cardiac imaging assessment such as echocardiography and cardiac magnetic resonance (i.e. right and left ventricular dysfunction, RVEF, LVEF) (14,15,21–30). Additionally, studies have reported results from invasive electrophysiological tests such as programmed ventricular stimulation (inducibility) or electro-anatomical mapping (presence of low voltage areas or areas of fractionated potentials) as useful for risk stratification in selected cohorts of patients with ARVC (31–33). By combining these predictors and the presence of previous sustained arrhythmic events, the 2015 International Task Force Consensus (ITFC), the 2017 American College of Cardiology (ACC) / American Heart Association (AHA) / Heart Rhythm

Society (HRS) guidelines, the 2019 HRS consensus, and the 2022 European Society of Cardiology (ESC) guidelines have provided expert recommendations on how to proceed with ICD placement in patients with ARVC (7–9,34) (**Figure 1**).

The ARVC Risk Calculator

While there is overwhelming consensus upon the clear benefits of offering ICDs to patients with ARVC that have experienced previous episodes of sustained VAs (7–9), the indication for primary prevention ICD placement in patients with ARVC have been extensively debated. Studies assessing the performance of these ICD placement indications, in fact, have reported limited effectiveness in risk stratification among patients without previous VA, with a high number of ICD implanted per sustained VA treated (29,35).

To better aid medical providers and patients in the primary prevention ICD decision making for patients with a definite diagnosis of ARVC and no prior sustained VA events, a risk stratification tool was proposed by a multinational collaboration in 2019 (29). This tool, called the ARVC Risk Calculator, leverages 7 clinical variables (age; sex; number of leads with a negative T wave on a 12 lead ECG; 24-h PVC burden; history of NSVT; history of a recent (<6 months) cardiac syncope episode; RVEF from cardiac magnetic resonance) to provide estimations for the 5-year risk of sustained arrhythmic events (a composite outcome including sustained ventricular tachycardias, ventricular fibrillation/flutter, arrhythmic sudden cardiac death episodes, and appropriate ICD therapies). The ARVC Risk Calculator was developed from a multicenter cohort of 528 patients from six countries and showed a good internal reliability with a bootstrapped C statistic of 0.77 [0.73–0.81]. A subsequent study from the same collaboration expanded the risk calculator for the estimation of the risk of rapid VA events (>250 bpm) (36). An online risk calculator reporting estimates from both models has been made available at www.arvcrisk.com. This risk stratification tool was well received by the ARVC scientific community several reasons: the clinical variables used are derived from clinical tests recommended by available guidelines and routinely collected in most

ARVC/cardiomyopathy clinics, rendering its use easy to implement (7,37). Additionally, its integrative approach allowed for a weighting, summarization, and normalization of multiple VA predictors, providing a single numerical output that could be used for informed decision-making conversations between patients and healthcare provides. Finally, at decision curve analysis, the ARVC Risk Score yielded a higher clinical benefit than the 2015 TFC consensus for ICD placement guidance, leading to the same patient protection rate from VAs but at the advantage of a 20.3% reduction in ICD placed.

Shortly after its publication, multiple independent study groups tested the performance of the ARVC Risk Calculator in external cohorts of patients with ARVC in Europe and Asia. A good performance of the risk calculator was observed in two cohorts of 88 primary prevention (38) and 140 mixed primary and secondary prevention ACM patients from Italy (27), although both studies reported the risk stratification tool underpredicting risk in patients with left ventricular involvement and in left dominant ARVC cases. Subsequent studies from France (115 primary prevention ARVC patients) (39) and China (88 mixed primary and secondary prevention ACM patients) reported similar results, showing high discriminatory performance for VA of the risk calculator in those patients for which the ARVC calculator was originally developed (Performance in primary prevention ARVC patients: *Baudinaud* et al C statistic 0.84 [0.74 – 0.93]; *Zhang* et al C statistic: 0.833 [0.615– 1.000]). All those studies, however, were hampered by relatively low sample sizes and a full scale validation of the ARVC Risk Calculator was achieved only in 2022, when two independent studies by *Protonotarious* and *Jorda* were simultaneously published (40,41). *Jorda* and colleagues supported the effectiveness and reliability of the ARVC Risk Calculator, reporting a good discrimination of the risk stratification tool (C statistic 0.70 [0.65–0.75]) in a large, multicentered cohort comprising of 429 ARVC patients enrolled from 29 centers in North America and Europe (41). The findings derived from a cohort of 554 ARVC patients led *Protonotarios* et al to similar conclusions (Overall C statistic: 0.75 [0.70 – 0.81]), while however observing a significant impact of the underlying genotype on the performance of the ARVC Risk Calculator (C statistic for gene-positive patients vs gene-elusive

patients: 0.82 [0.76–0.88) vs 0.65 [0.57–0.74]) and a potential risk overprediction of the tool for patients harboring a DSP pathogenic/likely pathogenic variant. **Table 3** lists all studies addressing the ARVC risk calculator that have been currently published.

REFINEMENT OF THE ARVC RISK CALCULATOR

Since its first development, several areas of potential improvement for the ARVC risk calculator were immediately identified (37). In the years following its development, numerous studies aimed to improve and refine the ARVC Risk Calculator by addressing the role of variables that originally were not included.

The Role of Physical Exercise

A first topic of discussion has been physical exercise, which is a well-known risk factor for patients with ARVC (42,43). Multiple studies have shown that physical exercise (and in particular endurance training) is associated with an increase in disease penetrance, arrhythmic risk, and adverse cardiovascular outcomes in patients with ARVC (44,45). A clear dose-response association between the quantity of physical exercise and the increase of risk has been shown (44,46), as well as a significant improvement in clinical parameters (RVEF, PVC burden, NSVT, and arrhythmia development during ECG stress test) and a lowering in VA rates risk during follow-up after de-training and exercise restriction (47,48). Due to these reasons, a diagnosis of ARVC represents an absolute contraindication to the competitive sports eligibility and patients with ARVC are recommended to limit the amount of exercise practice they entertain (7,8).

In the initial ARVC Risk Calculator, no risk estimate correction for exercise exposure was present and it was therefore questioned whether this tool would adequately perform in ACM patients with a high-dose exercise exposure. This question was first tested by *Gasperetti* et al in a cohort of 20 high-end endurance athletes diagnosed with ARVC. Although underpowered, in this cohort the ARVC risk calculator yielded a good risk stratification performance, with an almost perfect overlap

between predicted and observed risk was observed (47). These findings were later confirmed and expanded by a larger study performed by *Bosman* and colleagues (46), enrolling 176 definite diagnosis ARVC patients for which an exercise interview and a lifetime exercise exposure assessment were available. As expected, physical exercise clearly associated with a higher arrhythmic risk during follow up in this cohort. The ARVC risk calculator VA risk stratification performance, however, remained high (C statistic: 0.77 [0.71–0.84]) in all tertiles of exercise exposure (>18 METh/wk; >24METh/wk; >36METh/wk) and no significant improvement in model performance was shown when model integration of exercise dose-exposure was performed. Both studies postulated the status of athlete and a high-end exercise exposure to be strongly associated with at least 5 of the 7 variables included in the risk calculator (namely young age, higher PVC count, more TWI at 12-lead ECG, more NSVT, lower RVEF). Due to this association, the increased arrhythmic risk associated with physical exercise would therefore be already accounted for in the calculator, allowing its use in athletic or sedentary ARVC patients alike.

Advanced imaging and ventricular strain

In the modern era of advanced imaging, speckle tracking and myocardial strain assessments have gained momentum as potential additional risk predictors in numerous cardiomyopathies. ARVC represents no exception: in recent years, multiple reports have shown good association between a reduced myocardial strain and arrhythmic outcomes in this patient population (49–51).

The integration of those findings, however, with the more traditional risk assessment strategies, as well as the ARVC Risk Calculator, has not been attempted until very recently. A recent study from *Bourfiss* et al, in fact, investigated the prognostic value of RV and LV CMR-derived strain in a cohort of 132 patients with ARVC and no prior VA events and tested whether the integration of strain data in the ARVC Risk Calculator would improve its performance (52). In this study, both CMR-derived RV and LV strain were shown to be significantly associated with VA events occurring during follow-up in this patient cohort. However, both parameters lost statistical significance after

correcting for RVEF, LVEF, or the predicted arrhythmic risk derived from the ARVC Risk Calculator, therefore showing only a modest incremental value in risk stratification. Similarly, the performance of the ARVC Risk Calculator was not shown to improve significantly if the CMR-derived strain parameter with the strongest association with arrhythmic events (namely the LV global and septal circumferential strain) was added to the model (Pre and Post strain integration C statistic of the ARVC Risk Calculator: 0.76 [0.63–0.90] vs 0.82 [0.72–0.92], p=0.31). According to these findings, no additional prognostic value of speckle tracking seems to be present and no evidence seems to support the inclusion of speckle tracking assessment as a part of those cardiac instrumental tests routinely asked for ARVC risk assessment.

Programmed ventricular stimulation in primary prevention assessments

Another area of potential improvement for the ARVC risk calculator was the integration of the inducibility status retrieved from programmed ventricular stimulation (PVS). Over the years, the role of PVS for arrhythmic risk stratification in ARVC has been extensively debated, with some studies reporting a poor positive predictive value (53) and multiple others instead showing a significant role in the risk stratification process (15,28,54–57). Often, however, those studies have been hampered by small sample sizes and included a mix of patients with borderline and definite diagnosis of ARVC, as well as both patients with and without an history of previous sustained VA at the time of PVS performance. For these reasons, until recently, clear data addressing the utility of PVS in primary prevention for patients with ARVC were lacking.

A recent multicenter study from *Gasperetti* et al reported data from 288 patients with definite ARVC without a previous history of sustained VA undergoing PVS (33). Half of the study cohort resulted inducible for monomorphic ventricular tachycardia at PVS, and inducibility at PVS was shown a strong independent predictor of sustained VA during follow-up. Through a Bayesian analysis, the ARVC risk calculator a-priori derived risk was integrated with the PVS inducibility status, thus refining the 5-yr risk estimation and improving performance of the prediction model

(Combined C statistic: 0.75). Furthermore, although PVS status improved performance independently from the a-priori risk, the maximal benefit of PVS results was observed in patients with a low to moderate a-priori risk calculator derived risk (5-yr risk <25%). In this subset of patients, PVS yielded a high negative predictive value (92.6%) for VA, suggesting that non-inducibility at PVS could represent an additional factor for deciding against an ICD. The arvcrisk.com website has been updated to allow for individual calculation using this Bayesian approach.

Longitudinal Assessment of Arrhythmic Risk Over Time

The ARVC Risk Calculator was developed to provide 5-yr arrhythmic risk estimation for a baseline assessment and aid in the ICD decision making process at the time of disease diagnosis. ARVC, however, is a progressive condition, and the risk profile of patients with ACM may change over time, and the clinical predictors included in the ARVC Risk Calculator may be dynamic (30,58,59). Specifically, PVC count as well as NSVT at rest and during exercise have been shown to decrease at follow-up in patients with ACM, most likely due to exercise restrictions and use of medications (26,60). Furthermore, initiation of anti-arrhythmic medications and clinical detraining during follow up may change the VA risk of patients with ARVC (26,48,60,61). Additionally, "hot phases" of active disease and increased arrhythmic risk have been described during the natural history of this disease (62). It is therefore still of paramount importance to track and reassess ARVC patients multiple times during follow up, especially for those patients not deemed needing an ICD at baseline: the original arrhythmic risk assessment may not hold true after a few years of disease progression and patients initially at low arrhythmic risk may move towards higher risk groups, potentially benefitting of a second conversation regarding the need for an ICD.

The impact of repeated testing and longitudinal risk stratification in ARVC is, however, a relatively new topic of discussion in the field of ARVC. The use of 24-h Holters every 12-18 months to constantly reassess the arrhythmic risk of ARVC patients and their need for an ICD has only been recently described as an effective follow-up strategy (30). Changes in PVC count and NSVT at 24-

h Holter were showed to dynamically track the arrhythmic risk of patients with ARVC, with the overall PVC burden as well as its sudden increases (define as “PVC Spikes”) resulting associated with a strong arrhythmic risk increase over the year immediately following that Holter ECG. These data were recently confirmed and integrated by *Carrick* et al, reporting on the dynamic performance of the ARVC Risk Calculator as a whole during follow-up (60). This study showed that using the same baseline ARVC Risk Calculator estimates to assess risk at follow up evaluations resulted in decreased VA risk discrimination around the third year of follow up. This decrement, however, was completely negated by updating the ARVC Risk Calculator estimates feeding the algorithm with the most recent clinical value available during follow up examinations (i.e. repeated 24-h Holter examinations, echocardiograms, cardiac magnetic resonance scans), with the long-term risk stratification performance of such an updated model remaining high over the duration of follow up (C statistic ranging between 0.83 [0.80–0.86] and 0.79 [0.73–0.85]; **FIGURE2**).

While more prospective studies are clearly needed on this topic, no other data is currently available for longitudinal risk stratification strategies in ARVC. Similar findings are of paramount importance in everyday clinical practice because they provide guidance about how to integrate follow-up cardiac examinations into risk stratification strategies for patients with ARVC, extending beyond a simple baseline assessment. The use of the ARVC Risk Calculator for a dynamic risk assessment for primary prevention patients with ARVC seems effective and a risk stratification strategy employing full integration between this risk stratification tool and the clinical examinations recommended by current expert consensuses appears reasonable and easy to implement in the everyday clinical workflow of ARVC clinics.

COMPARISON OF THE ARVC RISK CALCULATOR WITH GUIDELINES

All the aforementioned studies have built a significant body of scientific evidence showing that the ARVC Risk Calculator is a reliable tool in risk stratification for the occurrence of VA events in patients with ARVC, both at baseline and during follow up examinations. The improvements and

sub-analyses performed over the years have extended its generalizability as well as the confidence in its predictions and in the most appropriate way of using it in clinical settings.

The decision of whether to use this tool in lieu of other stratification algorithms (i.e. the 2015 ITFC Consensus, the 2017 American Heart Association Guidelines for Sudden Cardiac Death, or the 2019 Heart Rhythm Society Consensus, the 2022 European Society of Cardiology Management of Ventricular Arrhythmia guideline) therefore lies in the relative increase in clinical benefit that this tool may have compared to those guidelines. In the original publication, the ARVC Risk Calculator was shown superior to the 2015 ITFC Consensus in terms of clinical net benefit (defined as number of ICD placed for treated event) at decision curve analysis regardless of the threshold used for recommending ICD implantation, reaching the same level of protection rate with an average 20.3% reduction in ICD implantation rate (29). A subsequent analysis from *Aquaro* and colleagues showed that an ARVC risk calculator 5-yr estimated risk threshold of 10% for ICD implantation achieved a higher protection rate and clinical net benefit than both 2015 ITFC and 2019 HRS recommendations (63). Similarly, in the patient cohort from *Casella* et al, an ARVC Risk Calculator derived 5-yr risk threshold ranging between 12.5% and 17.5% was identified as superior to the 2015 ITFC algorithm (38). The analysis from *Baudinaud* et al instead showed risk overestimation from the ARVC Risk Calculator for predicted risk estimates <50%; nonetheless, the ARVC Risk Calculator still outperformed the 2015 ITFC in their patient population (39). Finally, in the ARVC patient population used by *Jorda* et al for model validation, the ARVC Risk Calculator clinical benefit resulted superior to the 2015 ITFC, 2017 AHA, and 2019 HRS ICD placement recommendations at all given thresholds, with the ARVC Risk Calculator and the 2019 HRS performance becoming similar for 5-yr risk estimates of ~35% (41) (**FIGURE3**). This tool seems therefore to perform better for arrhythmic risk stratification in primary prevention patients with ARVC that all the currently available risk stratification guidelines. While no prospective data on its use is currently available, the same holds true for the other expert consensuses and statements dealing with this topic. Furthermore, this tool has been tested and found effective in a significant patient population (more than 1500

different ARVC patients combined) across different ARVC clinic (arrhythmic vs heart failure based) in different continents (Europe, America, and Asia).

One of the big unanswered questions is where a ARVC Risk Calculator derived risk threshold for recommending an ICD placement should lie. A risk threshold in the range of 5-25% 5-yr could be reasonable given the observed data. It is however important to remark that the final decision regarding primary prevention ICD implantation should always be taken through a patient-physician informed discussion. The ARVC Risk Calculator derived estimated risk is only one among many factors behind the ICD decision, with patients' preference and values representing the real deciding ones. The ARVC Risk Calculator should not replace the human interaction component nor the individual experience in ARVC patient management gathered by individual referral centers and specialized clinics. This tool is only meant to provide numerical guidance in the ICD decision making process and is to be integrated within a comprehensive and holistic clinical workflow.

CONCLUSION AND FUTURE DIRECTIONS

The current ARVC risk calculator as well as the other ARVC guidelines have been mainly focusing on patients fulfilling a definite diagnosis in accordance to the 2010 TFC for ARVC. Gene-elusive ARVC patients and patients with *PKP2* variants represent the majority of ARVC patients and fulfill 2010 TFC very often. The same however is true only for about half of patients carrying variants in genes such as *DSP*, *PLN*, and *FLNC* (64–67): patients with these genotypes represent a distinct ARVC subpopulation, with their biventricular or a left dominant phenotypes significantly differing from the classical RV dominant disease for which ARVC guidelines were developed. These genotypes are associated with a significant arrhythmic burden, nonetheless the most appropriate risk stratification strategies for those patients are still unclear: both analyses from *Casella* et and *Aquaro* et al reported a significant risk VA underprediction of the ARVC risk calculator in patients with a left-dominant ARVC phenotype (27,38), while a recent work from *Protonotarios* et al showed the

ARVC Risk Calculator overpredicting arrhythmic risk in ARVC patient with a pathogenic/likely pathogenic variants in the *DSP* gene fulfilling the conditions for ARVC Risk Calculator usage (40).

Due to the significant differences in presentation and phenotypes among patients grouped under the ARVC definitions, a phenotype-only risk stratification approach currently seems outdated. Among patients with a 2010 TFC phenotype, *Protonotarios* et al clearly showed the strong importance of the underlying genotype when assessing individual ACM patients' risk for VA (40). A recent study from *Paldino* et al even showed that a genotype-based classification of cardiomyopathies allows an improved long-term arrhythmic outcome stratification compared to a phenotype-based one among patients with genetically determined DCM and ACM phenotypes. (68) In this cohort, patients with *DSP*, *LMNA* and *FLNC* variants experienced consisted VA event rates regardless of the fulfillment of a 2010 TFC phenotype.

Clearly, more data characterizing the impact of genotype on arrhythmic events is needed, but we envision a shift towards an individual gene-based patient management rather than grouping patients largely by similar clinical phenotypes. Although we expect many of the VA predictors (i.e. NSVT, RV/LV dysfunction) to be shared across ARVC patients with different underlying genetic variants, their relative weight may vary and the role of some external stressors (i.e. physical exercise) may be different. Gene-specific algorithms have already been proposed with good results for some ARVC genotypes (66,67), as well as for other genetically determined cardiomyopathies(69), regardless of their phenotype. A precision medicine approach accounting for the genotype as well as for the clinical and structural characteristics of those diseases seems to be the upcoming future of the field of ARVC.

Table 1

Gene associated with ARVC

	Localization	Inheritance	ARVC Phenotype	Specific abnormalities	Dedicated Risk Stratification?
Plakophillin 2 (*PKP2*)	Desmosome	AD	Right Dominant	Highest susceptibility to exercise	No but prototype for (29)
Desmoplakin (*DSP*)	Desmosome	AD/AR	Biventricular or Left Dominant	Hair and skin features Myocarditis-like episodes	No
Desmoglein 2 (*DSG2*)	Desmosome	AD/AR	Biventricular		No
Desmocollin 2 (*DSC2*)	Desmosome	AD/AR	Right Dominant		No
Junction Plakoglobin (*JUP*)	Desmosome	AR	Right Dominant or Biventricular	Hair and skin features Naxos Disease	No
Desmin (*DES*)	Intermediate Filament	AD	Right Dominant	AV conduction disorders Skeletal myopathies possible	No
Transmembrane Protein 43 (*TMEM43*)	Nuclear Envelope	AD	Biventricular or Left Dominant	Extremely aggressive	No
Phospholamban (*PLN*)	Calcium Handling	AD	Biventricular or Left Dominant		Yes (67)

Table 2

Predictors at Baseline of Sustained Ventricular Arrhythmic Events (modified and integrated from Krahn et al (70))

First Author / Year	N of Patients	Predictor	OR/HR
Age			
Corrado (2003) (14)	132	Age (5-yr increase)	0.77
Orgeron (2017) (15)	312	Age < 30	3.14
Cadrin-Tourigny (2019) (29)	528	Age (1-yr increase)	0.98
Cadrin-Tourigny (2021) (36)	864	Age (1-yr increase)	0.96
Carrick (2022) (60)	408	Age (1-yr increase)	0.978
Sex			
Mazzanti (2016) (16)	301	Male	2.49
Martin (2016) (17)	26	Male	1.60
Lin (2017) (18)	70	Male	2.41
Cadrin-Tourigny (2019) (29)	528	Male	1.63
Cadrin-Tourigny (2021) (36)	864	Male	1.99
Carrick (2022) (60)	408	Male	1.746
*Protonotarios (*2022) (40)	554	Male	1.734
Exercise			
Mazzanti (2016) (16)	301	Exercise	2.98
Bosman (2022) (46)	178	Exercise >30 METh/wk	3.00
Cardiac Syncope			
Corrado (2010) (53)	106	Syncope	2.94
Battipaglia (2012) (71)	30	Unexplained Syncope	16.1
Mazzanti (2016) (16)	301	Syncope	3.36
Cadrin-Tourigny (2019) (29)	528	Cardiac Syncope < 6 m.o.	1.93
Carrick (2022) (60)	408	Cardiac Syncope < 6 m.o.	1.554
Protonotarios (2022) (40)	554	Cardiac Syncope < 6 m.o.	2.672
QRS			
Turrini (2001) (22)	60	QRS dispersion	1.22
Canpolat (2013) (25)	78	QRS interval fractionation	6.52
T wave inversion			
Cadrin-Tourigny (2019) (29)	528	N of leads with TWI	1.12
Cadrin-Tourigny (2021) (36)	864	N of leads with TWI	1.12
Carrick (2022) (60)	408	N of leads with TWI	1.10
Protonotarios (2022) (40)	554	N of leads with TWI	1.36
PVS			
Roguin (2004) (55)	42	PVS inducibility	11.2
Bhonsale (2011) (28)	84	PVS inducibility	4.50
Orgeron (2017) (15)	312	PVS inducibility	2.28
Casella (2020) (38)	101	PVS inducibility	8.9
Gasperetti (2022) (33)	288	PVS inducibility	2.52
Non-Sustained VT			
Bhonsale (2011) (28)	84	Non Sustained VT	10.50
Cappelletto (2018) (26)	98	Non Sustained VT	3.28
Cadrin-Tourigny (2019) (29)	528	Non Sustained VT	2.25
Gasperetti (2022) (30)	169	Non Sustained VT	2.29
Carrick (2022) (60)	408	Non Sustained VT	2.126

Protonotarios (2022) **(40)**	554	Non Sustained VT	1.36
EAM derived			
Santangeli (2012) (31)	32	Fragmented potentials	21.22
Migliore (2013) (32)	69	Low voltage areas	1.70
Lin (2017) (18)	70	Low potential areas	1,07
Casella (2020) (38)	101	Late fragmented potentials	7.4
SAEG			
Pezawas (2006) (24)	34	SAEG ≥ 2/3 parameters	45.04
PVC			
Orgeron (2017) (15)	312	PVC burden >1000/24h	4.43
Orgeron (2018) (23)	365	PVC burden >1000/24h	5.24
Cadrin-Tourigny (2019) (29)	528	(log) 24-h PVC burden	1.19
Cadrin-Tourigny (2021) (36)	864	(log) 24-h PVC burden	1.12
Gasperetti (2022) (30)	169	(log) 24-h PVC burden	1.50
Carrick (2022) (60)	408	(log) 24-h PVC burden	1.321
Protonotarios (2022) (40)	554	(log) 24-h PVC burden	1.167
RV alteration			
Turrini (1999) (72)	38	RVEF <50%	4.66
Wichter (2004) (21)	60	RV dysfunction	2.09
Canpolat (2013) (25)	78	RVEF reduction	3.76
Cappelletto (2018) (26)	98	RV FAC (1% increase)	0.35
Cadrin-Tourigny (2019) (29)	528	RVEF (1% decrease)	1.03
LV alteration			
Corrado (2003) (14)	132	LVEF	0.94
Pezawas (2006) (24)	34	LVEF reduction	1.20
Canpolat (2013) (25)	78	LV involvement	2.88
Aquaro (2020) (27)	140	LV involvement	4.20
Aquaro (2020) (27)	140	LV-dominant phenotype	3.40
Miscellanea			
Battipaglia (2012) (71)	30	RR variabilitiy in the LF amplitude	0.88
Mazzanti (2016) 30)	301	History of Atrial Fibrilaltion	4.38

Only studies reporting a) a measure of association with arrhythmic events and b) patients with a definite diagnosis of ARVC have been included in this table

Table 3

External Validation Studies

	Patients (n)	Follow up (years)	Events (n/%)	Findings	Comments
Casella et al (38) (2020)	82	5.41 [2.59–8.37]	28 (34.1)	Good performance of Risk Calculator in classic ARVC forms	Risk Calculator underpredicts risk in BiV/LD forms
Gasperetti et al **(47)** (2020)	20	5.3 [3.2–6.6]	6 (30.0)	Good performance of Risk Calculator in ARVC patients with a high exercise exposure	Very high-end endurance athlete cohort
Aquaro et al (27) (2020)	140	5.0 [2.0–8.0]	48 (34)	Good performance of Risk Calculator in classic ARVC forms	Mix of primary/secondary prevention pts; Risk Calculator underpredicts risk in BiV/LD forms
Baudinaud et al (39) (2021)	115	7.8 [6.1–9.7]	15 (12)	C statistic: 0.84 (0.74–0.93)	Risk overestimation for low risk patients
Zhang et al (73) (2022)	88	3.9 [1.6–6.9]	57 (64.8)	Overall C statistic: 0.681 (0.567–0.796) Primary Prevention C statistic: 0.833 (0.615–1.000) Secondary Prevention C statistic: 0.640 (0.510–0.770)	Mix of primary and secondary prevention pts
Protonotarios et al (40) (2022)	554	6.0 [3.1 – 12.5]	100 (18)	Overall C statistic: 0.75 (0.70–0.81) Gene-positive C statistic: 0.82 (0.76–0.88) Gene-elusive C statistic: 0.65 (0.57–0.74) *PKP-2* C statistic: 0.83 (0.75–0.91) *DSP* C statistic: 0.80 (0.53–0.96)	Significant impact of genotype on Risk Calculator performance
Jorda et al (41) (2022)	429	5.02 [2.05–7.90]	103 (24)	C statistic: 0.70 (0.65–0.75)	-

Additional Calculator Refinements

	Patients (n)	Follow up (years)	Events (n/%)	Findings	Comments
Bosman et al (46) (2022)	176	5.4 [2.7–9.7]	54 (30.7)	C statistic: 0.77 (0.71–0.84)	No need for exercise correction in the Risk Calculator estimates
Gasperetti et al (33) (2022)	288	5.31 [2.89–10.17]	83 (60.6)	Integrated C statistic of Risk Calculator + PVS: 0.75	Maximal benefit of PVS performance in moderate risk patients (<25% 5-yr predicted risk) for ICD placement exclusion
Bourfiss et al (52) (2022)	132	4.3 [2.0–7.9]	25 (19.0)	C statistic Risk Calc: 0.76 [0.63–0.90] Integrated C statistic Risk Calc + LV strain: 0.82 [0.72–0.92]	Inclusion of CMR derived LV global and septal circumferential strain does not improve the model

Figure 1

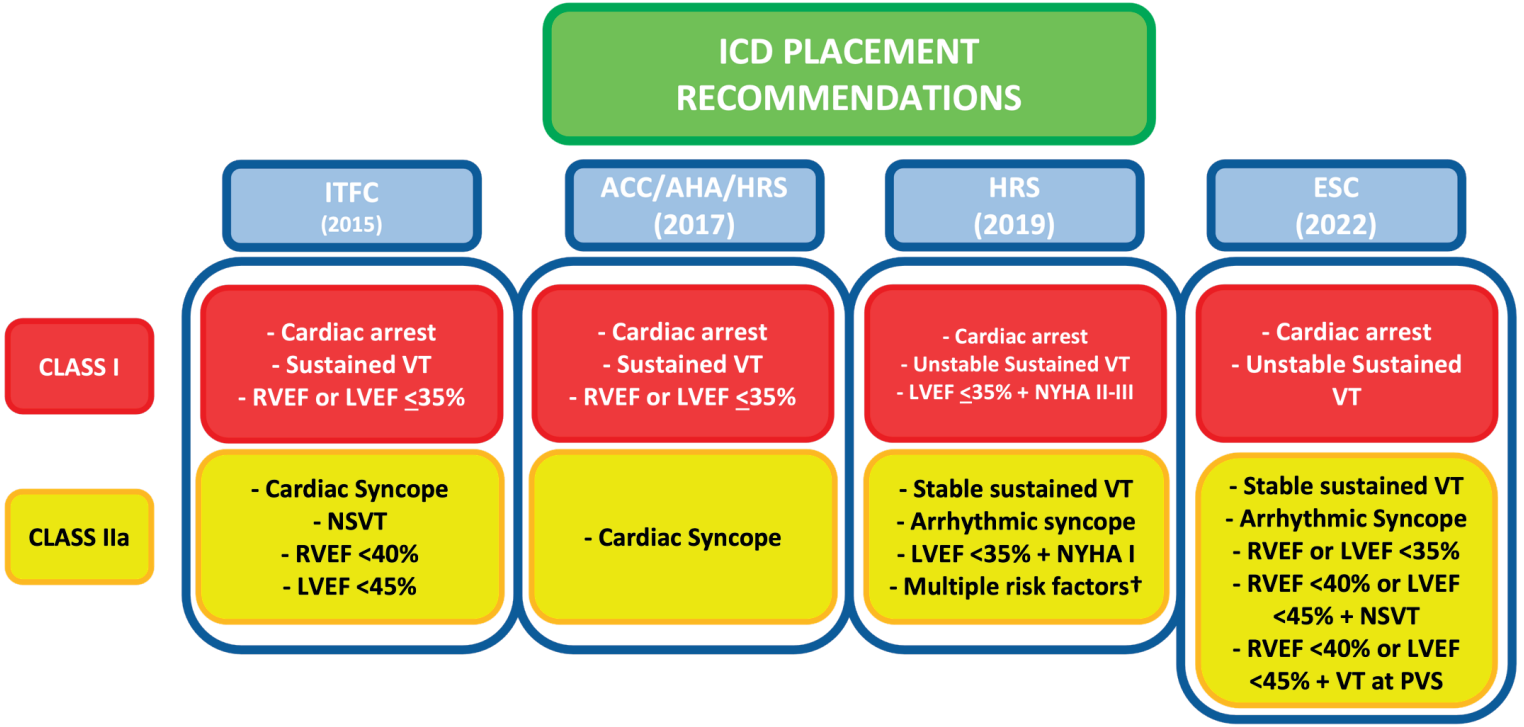
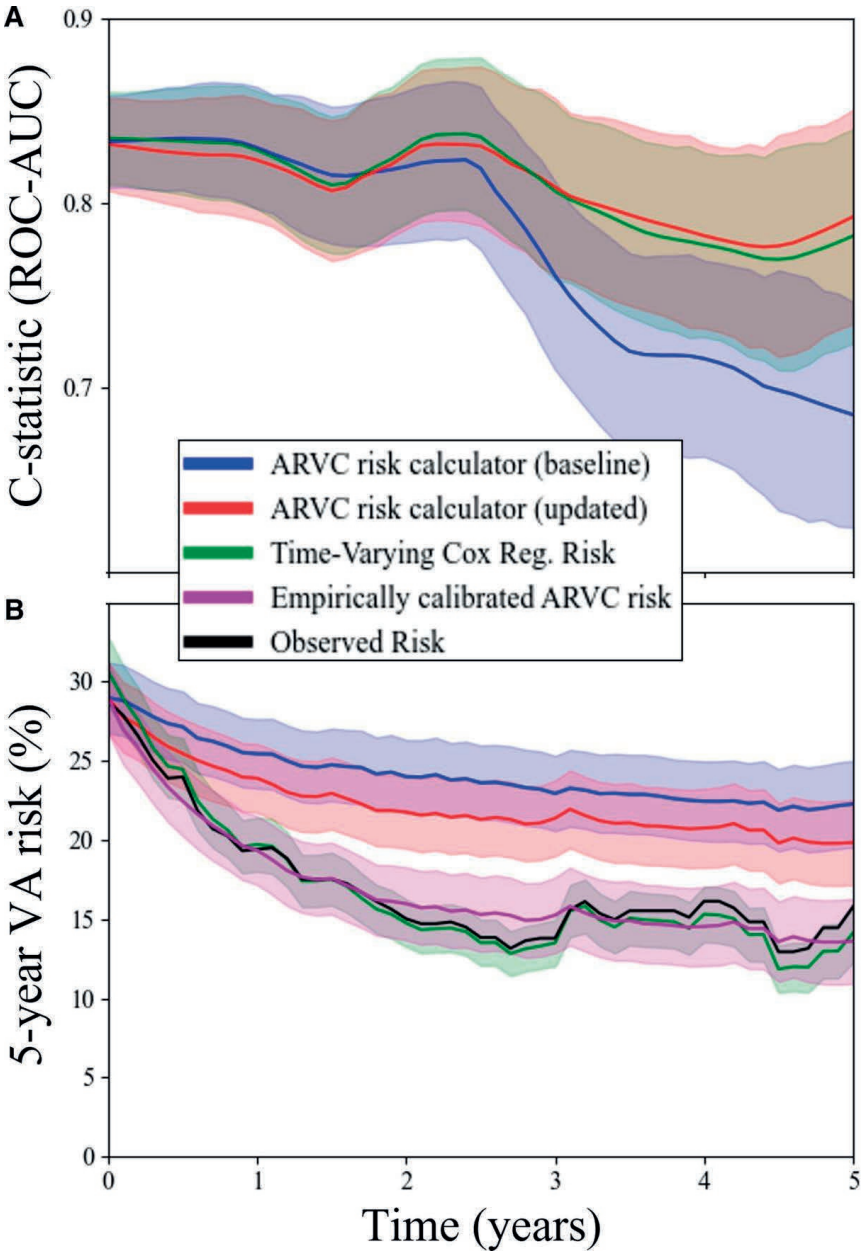


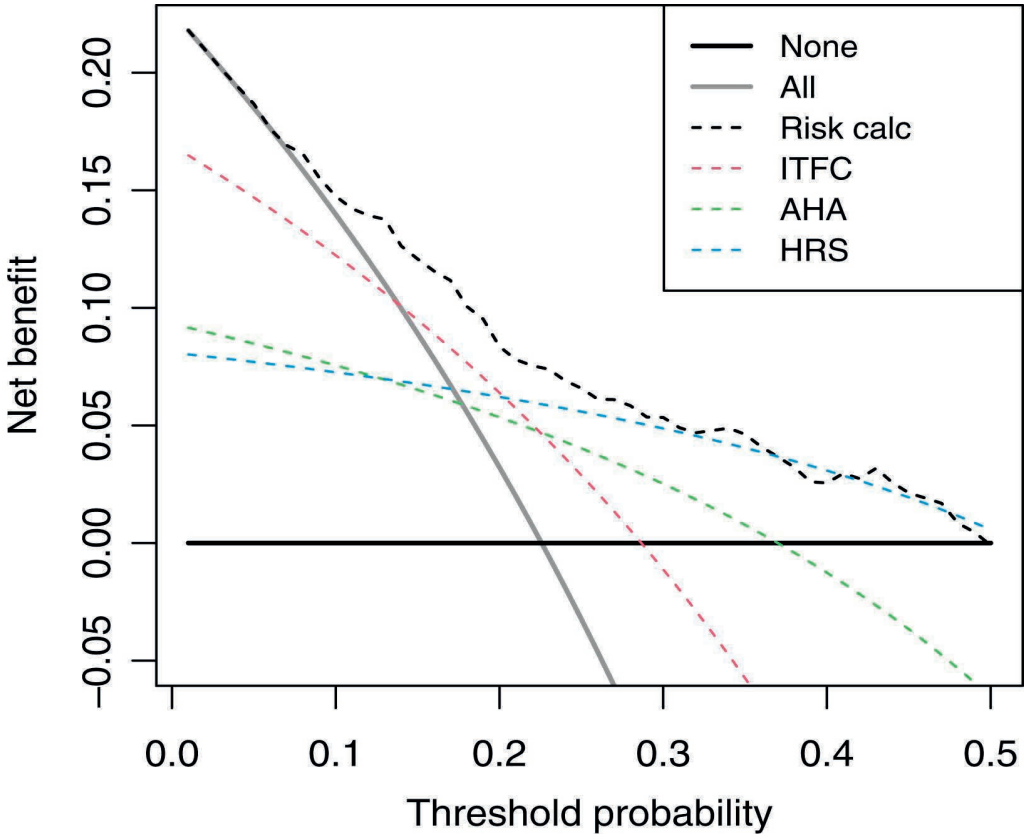
Figure 1 reports the different indications for ICD placement in patients with ARVC in a) the 2015 International Task Force Consensus for ARVC; b) the American College of Cardiology / American Heart Association / Heart Rhythm Society 2017 Guidelines; c) the 2019 Heart Rhythm Society Consensus for Arrhythmogenic Cardiomyopathy; and d) the 2022 European Society of Cardiology Guidelines for Ventricular Arrhythmias

Figure 2



Long term performance of the ARVC Risk Calculator for VA during follow up (Reproduced from Carrick et al – Chapter 6); the performance of the non-updated ARVC Risk Calculator drops around the third year of follow up (blue line in the top panel). This drop in performance is negated if the ARVC Risk Calculator is updated with the most recent set of clinical tests (red line)

Figure 3



Decision curves showing superior net clinical benefit of an ICD placement strategy using the ARVC Risk Calculator compared to the 2015 International Task Force Consensus for ARVC (ITFC); b) the American College of Cardiology / American Heart Association / Heart Rhythm Society 2017 Guidelines (AHA); c) the 2019 Heart Rhythm Society Consensus for Arrhythmogenic Cardiomyopathy (HRS) for any threshold below 35% of 5-yr predicted risk. For any threshold above 35%, the risk stratification performance of the HRS consensus approximates the ARVC Risk Calculator. (Reproduced by Paloma et al – Chapter 4)

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PART I

VALIDATION OF THE ARVC RISK CALCULATOR

CHAPTER 3

LONG-TERM FOLLOW-UP ANALYSIS OF A HIGHLY CHARACTERIZED ARRHYTHMOGENIC CARDIOMYOPATHY COHORT WITH CLASSICAL AND NON-CLASSICAL PHENOTYPES - A REAL-WORLD ASSESSMENT OF A NOVEL PREDICTION MODEL: DOES THE SUBTYPE REALLY MATTER?

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Europace. *2020 May 1;22(5):797-805. doi: 10.1093/europace/euz352.PMID: 31942607*

ABSTRACT:

Aims: to provide long term outcome data on arrhyhtmogenic cardiomyopathy (ACM) patients with non-classical forms (Left Dominant ACM (LD-ACM) and Biventricular ACM (Bi-ACM)) and an external validation of a recently proposed algorithm for ventricular arrhythmia (VA) prediction in ACM patients.

Methods: demographic, clinical, and outcome data were retrieved from all ACM patients encountered at our institution. Patients were classified according to disease phenotype (R-ACM; Bi-ACM; LD-ACM). Overall and by phenotype long term survival were calculated; the novel *Cadrin-Tourigny et al* algorithm was used to calculate the a-priori predicted VA risk, and it was compared with the observed outcome to test its reliability.

Results: one-hundred and one patients were enrolled; three subgroups were defined (R-ACM, n=68; Bi-ACM, n=14; LD-ACM, n=19). Over a median of 5.41 [2.59–8.37] years, the non-classical form cohort experienced higher rates of VAs than the classical form (5yr-freedom from VAs: 0.58 [0.43-0.78] vs 0.76 [0.66–0.89], p=0.04). The *Cadrin-Tourigny et al* predictive model adequately described the overall cohort risk (Mean Observed – Predicted Risk Difference (O-PRD): +6.7 [-4.3;+17.7] %, p=0.19); strafing by subgroup, excellent goodness of fit was demonstrated for the R-ACM subgroup (Mean O-PRD p=0.99) while in the Bi-ACM and LD-ACM ones the real observed risk appeared to be underestimated (Mean O-PRD: -20.0 [-1.1;-38.9] % , p <0.0001; -22.6 [-7.8;-37.5] %, p <0.0001 respectively).

Conclusion: Non-classical ACM forms appear more prone to VAs than classical forms. The novel prediction model effectively predicted arrhythmic risk in the classical R-ACM cohort, but seemed to underestimate it in non-classical forms.

INTRODUCTION:

Arrhythmogenic cardiomyopathy (ACM) is a heritable cardiac disorder characterized by fibro-fatty myocardial replacement, associated with an increased risk of ventricular arrhythmias (VAs) and sudden cardiac death (SCD). Historically, the right ventricle has been considered the first chamber affected by fibro-fatty replacement; recently however, mainly due to genetic testing and cardiac magnetic resonance (CMR) greater accessibility, there has been an increase in the number of diagnosis of non-classic ACM forms, with several reports of biventricular or left ventricular early disease involvement(1–5).

To date, no definitive ACM treatment is available: patient management has been focusing on arrhythmic risk stratification and VAs/SCD prevention. The International Task Force Consensus (ITFC) recognizes a pivotal role for implantable cardioverter-defibrillators (ICDs) in secondary prevention, but a worldwide accepted strategy for primary prevention has yet to be validated(6). How to stratify ACM arrhythmic risk in patients without major arrhythmic presentations has represented a clinical conundrum for decades: despite having identified several independent VAs predictors, until recently no unifying theory was available and the number of ICD placed appeared greatly exceeding their real need(7–10).

Recently, *Cadrin-Tourigny et al,* proposed a novel algorithm for ACM arrhythmic risk stratification; their model was proven superior to the ITFC model both in VAs prediction as well as in ICDs placement guidance(11). Although representing a major breakthrough in ACM management, this algorithm still requires real world validation, and its performance in ACM subtypes needs to be assessed.

This study aims to:

1) Describe the long-term clinical findings and outcomes in a large invasively-studied ACM patient cohort, including classic and non-classic forms;

2) Provide a validation to *Cadrin-Tourigny et al* novel model by assessing its reliability in an external cohort;

3) Assess *Cadrin-Tourigny et al* model reliability in non-classic ACM forms;

4) Analyze the predictivity of other invasive parameters non included in the novel model.

METHODS:

Patient population:

The study cohort was extracted from an ACM pathology registry at Centro Cardiologico Monzino, IRRCS (Milan, Italy); all consecutive patients with an ACM diagnosis undergoing invasive diagnostic tests were enrolled in the study. This analysis was approved by the local ethic review board and complies with the Declaration of Helsinki.

Arrhythmogenic Cardiomyopathy Diagnosis and Therapeutic Work-Up:

ACM diagnosis was postulated by a dedicated heart team composed of cardiac radiologists, electrophysiologists, and cardiac pathologists, in accordance with the 2010 Revised Task Force Criteria(12).

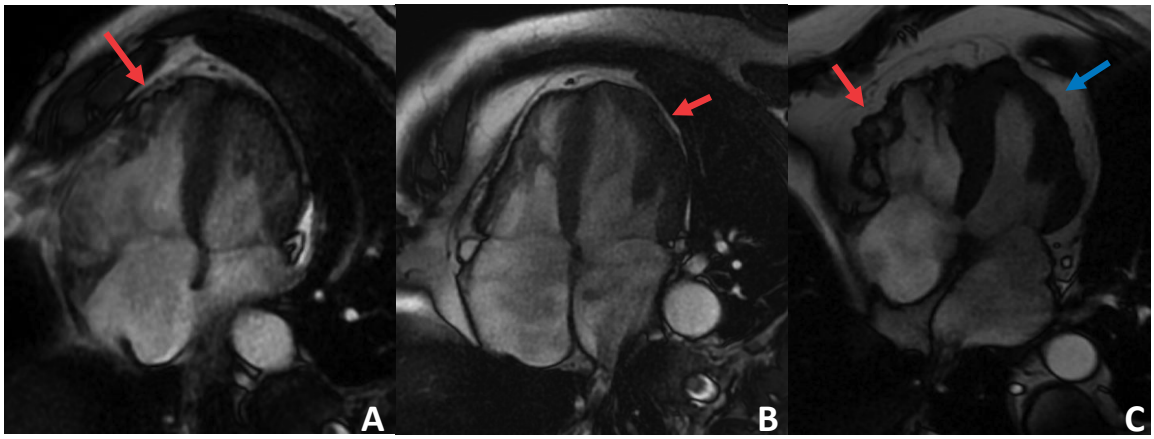
All the atypical ACM forms included reached at least a "borderline" level of diagnosis for the disease and presented further additional features of suspect of atypical ACM disease, including:

- A fibrofatty endo-myocardial biopsy fulfilling 2010 ITFC criteria performed in the left ventricle;
- A non-sustained or sustained ventricular arrhythmia of left ventricle origin morphology;
- A T wave inversion in left precordial (V4-V6) ECG leads;
- A left ventricular fibro-fatty replacement visualized at CMR dedicated evaluation as per reports and technique previously described (1,3); a definition of early left ventricle involvement was used to describe this CMR pattern when in absence of contextual right ventricle fibrofatty infiltration, motion and/or contractility abnormalities.

All patients fulfilling these criteria presenting a history of dilated cardiomyopathy or of a structural heritable cardiac diseases in the family other than ACM or with a genetic mutation clearly associated with dilated cardiomyopathy pathogenesis were enrolled nor in the registry neither included the analysis, in order to remove possible phenocopies.

Depending on disease presentation and ventricles involvement, patients were classified as follows: 1) classical ACM (R-ACM): predominant fibro-fatty infiltration of the right ventricle with no or late left ventricle involvement; 2) non classical ACM, further divided into 2a) biventricular ACM (BI-ACM): concomitant fibro-fatty infiltration of both ventricles at disease presentation; and 2b) left dominant ACM (LD-ACM): fibro-fatty infiltration of the left ventricle without right ventricle involvement. **[Figure1]**

FIGURE 1



Different ACM phenotypes are presented in the figure. In panel A, a classic form of R-ACM with right ventricular involvement (RV dilation with systolic bulging, red arrow); in panel B, a LD-ACM is presented with fibro-fatty infiltration of the posterolateral LV wall (red arrow); in panel C, a Bi-ACM is characterized by both right ventricular bulging (red arrow) and fibro-fatty LV infiltration (blue arrow). All images are cine b-SSFP.

Baseline and Non Invasive Evaluation:

Before hospital admission or during hospital in-stay before any invasive procedure, all patients underwent a routine evaluation, comprising of: 12-lead baseline ECG; complete blood panel; ACM

dedicated cardiac ultrasound and cardiac magnetic resonance (CMR). CMR protocol is reported in *Appendix1*. Genetic analysis for ACM related genes was performed on blood samples upon physicians' request.

Invasive Evaluation:

A baseline programmed electrical stimulation (PES) to assess arrhythmic inducibility, as well as a three dimensional (3D) endocavitary electro-anatomical mapping (EAM) were routinely performed in all patients; ventricular chamber endocardium and/or epicardium were explored and mapped accordingly to CMR findings and presumed site of origin of the arrhythmias. Upon physician indication, a percutaneous right ventricular endo-myocardial biopsy (EMB) was performed, possibly EAM guided, following previously described protocols and guidelines for ACM diagnosis. All bioptic samples were processed and evaluated c/o an high volume center; inflammatory cells and cardiotropic virus genome assessment on myocardial samples was routinely performed in all biopsies. SCD risk stratification analyses were performed in all patients as per the ITFC Statement for ACM treatment(6), and ICD placement performed accordingly. The need for anti-arrhythmic drugs (AADs) was evaluated in all patients and AADs eventually started after an electrophysiologist consult; trans-catheter ablation (TCA) was performed on a single-case base after physician evaluation with an endocardial, epicardial or endo/epicardial approach adequate to clinical/EAM findings.

Arrhythmic Events Evaluation and Follow Up:

At disease diagnosis, the 24-hour premature ventricular complex (PVC) burden and all complex arrhythmic events [Non-sustained ventricular tachycardia (NSVT); sustained ventricular tachycardia (SVT); ventricular fibrillation/flutter (VF)] at baseline and in patient history were assessed.

Clinical follow up was provided in all patients at 3-, 6-, and 12-months after disease diagnosis, and every 12 months thereafter, or immediately upon the occurrence of any complex arrhythmic event. A 24-hour Holter ECG test was required per institution protocol at every follow up visit. In case of ICD placement, device interrogation was performed every 6-8 months by dedicated physicians and a quick summary check was performed at every clinical follow up visit.

Study Outcomes:

The primary outcome for survival analysis was the first sustained VA event; sustained VAs were defined as a composite of SCD, SVT, VF or appropriate ICD intervention. The expected rate of VAs was calculated with the *Cadrin-Tourigny et al* predictive model in patients complying to model proprieties(11), and compared to the observed rate during follow up at fixed time intervals for both the overall cohort and different ACM subtypes; a retrospective "net benefit" analysis on *Cadrin-Tourigny* model impact on ICD placement was performed as well; all patients undergoing a TCA before any VA event during follow up were excluded from these sub-analysis. The possible VA predictive value of invasive parameters not included in the *Cadrin-Tourigny et al* algorithm (Namely: Inducibility at PES; retrieval of late potential at EAM; inflammatory infiltration and viral genome retrieval at EMB) was also assessed.

Statistical Analysis:

All statistical analyses were performed using R Project for *statistical computing* version 3.5. Continuous variables were expressed as mean±standard deviation or as median [interquartile range (IQR)], while categorical variables were expressed as counts (%); comparisons were performed using the independent sample t-test or the Mann-Whitney U test, as appropriate. Kaplan-Meier analysis with log-rank test were used to make statistical inference on long term outcome data. Predicted and observed

frequencies for each ACM subtype (R-ACM, Bi-ACM, LD-ACM) were modeled as a nested multivariate linear regression. The post-hoc analysis was corrected with the use of Tukey's correction. The findings were considered statistically significant with a two-tailed p value < 0.05. The real world model impact on ICD implant was evaluated through a "net benefit" assessment, similar to the Cadrin-Tourigny sub-analysis(11). Association between VA events and invasive parameters was tested with a multivariate logistic regression and the coefficients expressed as odds ratio with 95% confidence interval.

RESULTS:

Cohort Overview

The study cohort comprised of 101 patients undergoing invasive evaluation; the male to female ratio in our population was close to 3:1 (n=76 males; n=25 females), with age at diagnosis of 41.3±14.2 years. Eighty-four (87.1%) patients were probands, with only 17 (13.9%) referred family members; when assessing family history in probands, a history of SCD was present in 27 patients (32.1%). Of note, 19 (18.9%) patients were athletes, referred for a third-level evaluation.

A genetic analysis was performed in 59 (58.4%) patients, of which 36 (61%) tested positive for an ACM-likely pathogenic variant; among those, *PKP2* was the most commonly mutated gene [n = 18 (50.0%)]. At CMR, LVEF and RVEF values resulted 51.5±10.6 and 46.9±8.8, respectively.

Ninety-nine (99%) patients underwent complete invasive evaluation: thirty-five (35.4%) patients developed VAs (n=32 SVTs; n=3 VFs) at PES. Areas of low bipolar and unipolar potential mapping were visualized at EAM in 57 (57.6%) and in 66 (66.7%) patients, respectively, while late fragment potentials were found in 40 (40.4%). A percutaneous EMB was performed in 60 (59.4%) pts, with evidence of fibro-fatty replacement in 45 of them (75%). While 18 (30%) EMBs were performed before the introduction of the EAM-guided protocol, 42 (70%) were EAM guided. Pathological fibro-fatty

infiltration was present in 55% and 84% of the samples, respectively, showing a clear improvement in EMB performance when substrate-guided(13).

At disease diagnosis, history and documentation of sustained VAs was found in 15 (14.9%) patients, in half of which represented disease first clinical presentation. AADs were started in 41 (40.6%) patients, while other b-blockers than sotalol were employed in 47 (46.7%) patients. Finally, according to ITFC arrhythmic risk stratification, 68 (67.3%) patients underwent ICD implant, 8 (11.8%) of which were subcutaneous ICD. After completing diagnostic work-up, ACM dominance was assessed and patients were sub-classified as follows: n=68 (67.3%) R-ACM; n=14 (13.9%) Bi-ACM; n=19 (18.8%) LD-ACM. A complete list of the study population characteristics has been reported in **Table1**, both as overall and stratified by sub-groups.

Table I Baseline Population Characteristics

	Overall	**R-ACM**	**BI-ACM**	**LD-ACM**
Total	101(100)	68(67.3)	14(13.9)	19(18.8)
Demographics Characteristics				
Age at Diagnosis, years	41.3±14.2	39.5±13.8	39.4±11.6	49.2±15.4
Male, n	76(75.3)	51(75.0)	10(71.4)	15(79.0)
Proband, n	84(83.2)	57(83.8)	12(85.7)	15(78.9)
Recent Cardiac Syncope, n	13(12.9)	9(13.2)	2(14.3)	2(10.5)
History of CAE, n	15(14.9)	14(20.6)	0	1(5.3)
Genetic Analysis, n	59(58.4)	39(57.4)	8(57.1)	12(63.2)
Mutation, n	36(61.0)	21(53.8)	4(50.0)	11(91.7)
PKP-2, n	*18(50.0)*	*13(61.9)*	*2(50.0)*	*3(27.3)*
DSP, n	*9(25.0)*	*2(9.5)*	*1(25.0)*	*6(54.5)*
DSG-2, n	*3(8.3)*	*1(4.8)*	*1(25.0)*	*1(9.1)*
DSC, n	*1(2.8)*	*1(4.8)*	*0*	*0*
TMEM-43, n	*3(8.3)*	*3(14.2)*	*0*	*0*

Multiple Mutations, n	*2(5.6)*	*1(4.8)*	*0*	*1(9.1)*
Baseline and Holter ECG				
TWI in ≥3 precordial leads, n	45(44.6)	30(44.1)	8(57.1)	7(36.8)
TWI in ≥2 inferior leads, n	23(22.8)	9(13.2)	4(28,6)	10(52.6)
Epsilon Wave, n	7(6.9)	5(7.4)	2(14.3)	0
24hPVC burden, n	900 [300 – 2263]	1000 [300 – 2760]	700 [478 – 1501]	1200 [200 – 1707]
CMR Imaging				
RVEF, %	46.9±8.8	45.8±7.2	40.8±8.7	55.5±8.2
LVEF, %	51.5±10.6	54.9±8.9	46.3±11.4	43.3±10.2
Invasive Evaluation Data				
PES inducibility, n [*2]	35(35.4)	23(33.8)	3(21.4)	9(47.4)
SVT, n	*32(91.4)*	*22(95.6)*	*2(66.6)*	*8(88.9)*
VF, n	*3(8.6)*	*1(4.4)*	*1(33.3)*	*1(11.1)*
Low EAM Unipolar Potentials, n [*2]	66(66.7)	44(66.7)	11(78.6)	11(57.9)
Low EAM Bipolar Potentials, n [*2]	57(57.6)	37(56.0)	10(71.4)	10(50.2)
Late Fragmented EAM Potentials, n [*2]	40(40.4)	25(37.9)	7(50.0)	8(42.1)
EMB, n	60(59.4)	38(55.9)	8(57.1)	14(73.7)
Fibro-Fatty Infiltration, n	*45(75.0)*	*26(68.4)*	*7(85.5)*	*12(85.7)*
Inflammatory Infiltration, n	*12(20)*	*7(18.4)*	*2(25.0)*	*3(21.4)*
Viral Genome Detection, n	*8(13.3)*	*4(10.5)*	*1(12.5)*	*3(21.4)*
Treatment at Baseline				
ICD, n	68(67.3)	45(66.2)	11(78.6)	12(63.1)
Subcutaneous ICD, n	*8(11.8)*	*5(11.1)*	*0*	*3(25.0)*
AADs, n	41(40.6)	23(33.8)	7(50.0)	11(57.9)
B-Blockers, n	47(46.5)	25(36.8)	8(57.1)	14(73.7)
TCA at presentation, n	14(13.9)	12(17.6)	0	2(10.5)

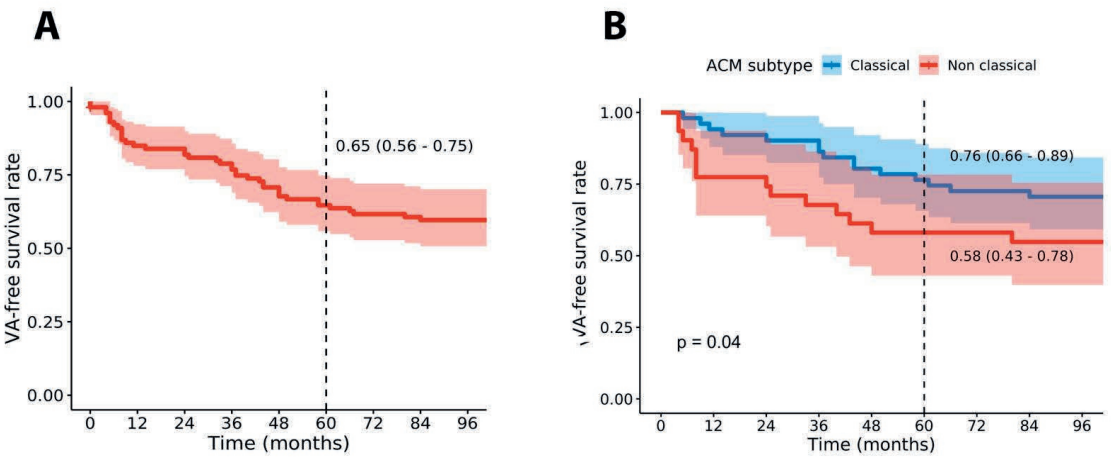
*Variables are expressed as counts (%), mean ± standard deviation, or median (IQR). * followed by a number indicates the number of patients for which that data was not available.CAE: complex arrhythmic Events (SVT and/or VF); DSP: desmoplakin; DSG-2: desmoglein-2; DSC: desmocollin; EAM: electro-anatomical mapping; EMB: endo-myocardial biopsy; CMR: cardiac magnetic resonance; PES: programmed electrical stimulation; PKP-2: plakophilin-2; PVC: premature ventricular complex; TMEM-43: transmembrane protein-43; TWI: T wave inversion; SVT: sustained ventricular arrhythmia; VF: ventricular fibrillation;*

Follow Up Analysis

Primary Outcomes

Over a median follow up of 5.41 [2.59–8.37] years, a first sustained VA event was documented in 43 (42.6%) patients, stratified as follows: in 28 (27.7%) patients an appropriated ICD intervention was observed, in 10 (9.9%) spontaneous SVT while in 4 (3.9%) SCD represented disease presentation and lead to disease diagnosis.The cumulative freedom from VA events of the whole cohort has been presented in **Figure2a** [Overall 5yr/freedom-from-VA rate 0.65 (0.56–0.75)]. A survival analysis for those patients in which the *Cadrin-Tourigny et al* algorithm was used has been reported in **Figure2b**, stratified by Classical and Non-Classical forms; at such analysis, Non-Classical forms outcome resulted significantly (p=0.04) worse than Classical (Classical Form 5yr/freedom-from-VA rate 0.76 [0.66–0.89]; Non-Classical Form 5yr/freedom-from-VA rate 0.58 [0.43–0.78])

Figure 2

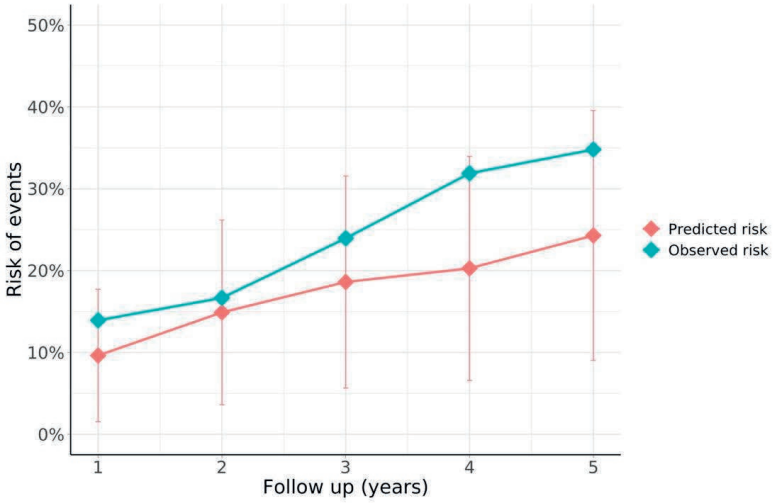


Cumulative freedom from sustained ventricular arrhythmias in overall cohort (A) and sub-cohorts (B). The 95% confidence intervals (shaded area) is reported.

Algorithm Predictivity Assessment

The predicted risk from *Cadrin-Tourigny et al* algorithm was calculated in 82 (81.2%) patients; a complete listing of this subpopulation characteristics has been reported in *Supplementary Table S1*. Predicted and observed VA rates over time for the overall population are reported in **Figure3**. Although a signal in difference can be qualitatively observed, observed rates for the overall sub-cohort resulted within the 95% CI of predicted rates and therefore non-different (Mean Difference Observed-Predicted Rate +6.7% [-4.3; +17.7] p=0.19), at all follow up times. **Figure4** (Representative Figure) reports the difference between predicted and observed rates stratified by ACM subtype: at the post-hoc analysis no significant difference between the predicted and observed arrhythmic event rates were assessed in the R-ACM subgroups (p=0.99); the observed rate instead exceeded the predicted one at mid/long term follow up in the Bi-ACM and L-ACM subgroups (Mean Difference Observed-Predicted: -20.0% [-1.1;-38.9], p<0.0001; -22.6% [-7.8;-37.5] p<0.0001, respectively). The complete risk analysis at different times with the time corresponding VA rates have been reported in *Supplementary Table S2*, for both the overall population and by subgroups.

Figure 3

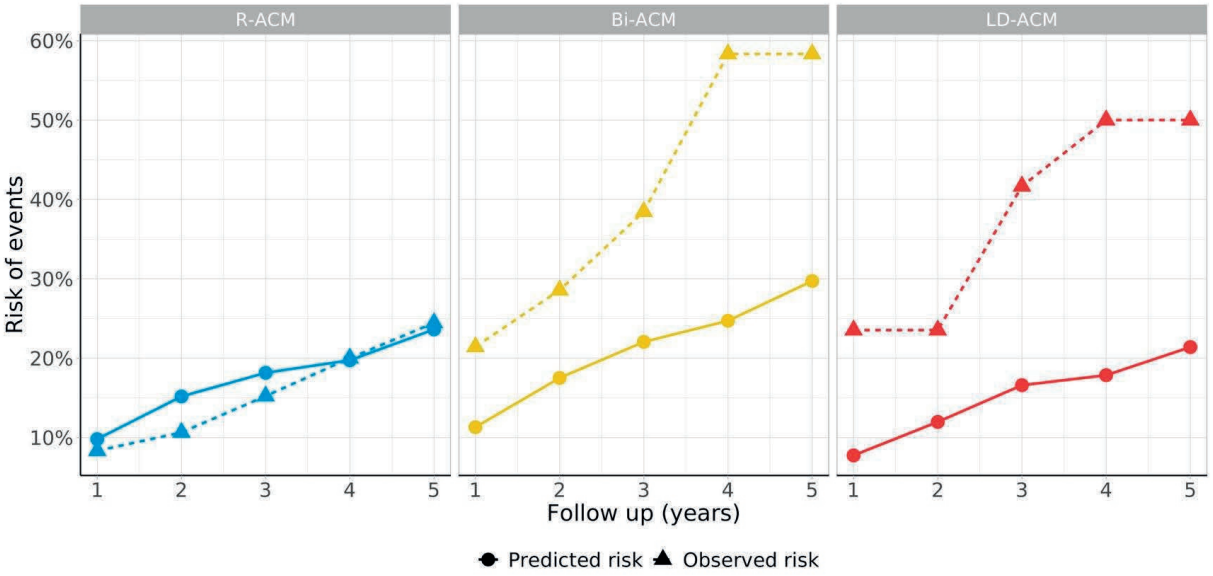


Year by year model predicted arrhythmic risk with related confidence interval (red) and the observed risk (blue) for the overall cohort.

Algorithm Clinical Impact

According to the ITFC risk assessment model, 52 (63.4) patients of the subpopulation underwent ICD implant. The impact of 5-year risk thresholds for ICD implantation with the *Cadrin-Tourigny et al* model vs the ITFC algorithm was assessed for the R-ACM sub cohort, given the best model fitness, and reported in **Figure5**; the *Cadrin-Tourigny et al* model guided ICD-placement method appeared to the ITFC consensus for 5-year risk thresholds between 15% (Same Net Benefit, better overall protection) and 20% (Better Net Benefit, same overall Protection). Complete sub-analyses have been reported in *Supplementary Tables s3-s3C and related figures* for completeness.

Figure 4 (Representative Figure)



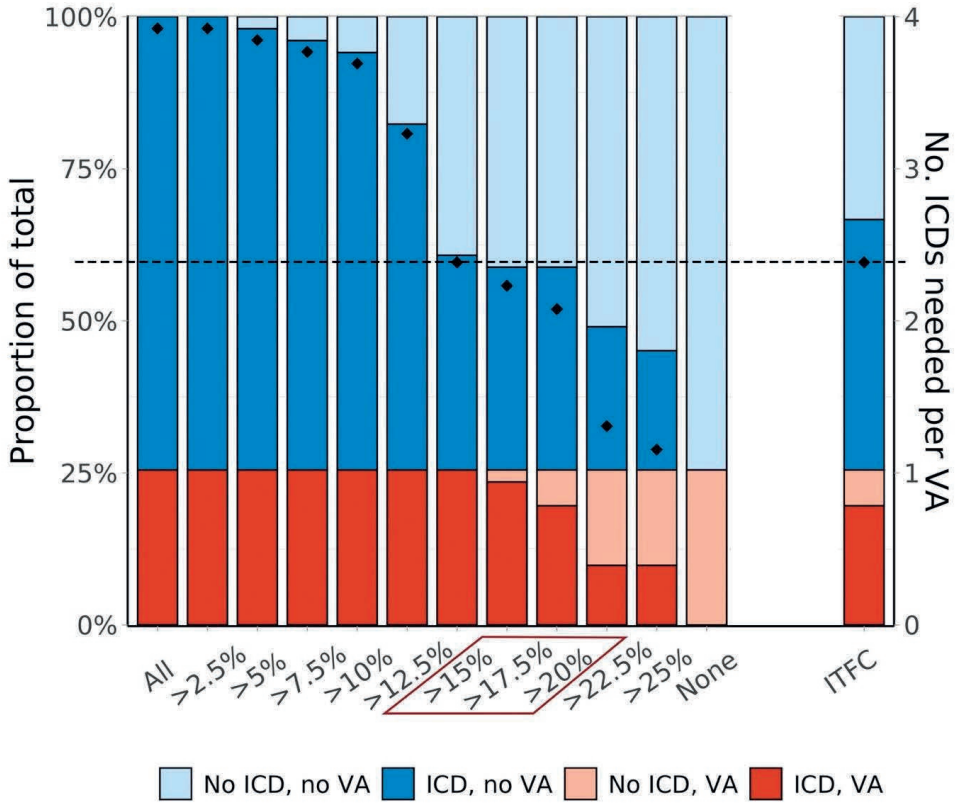
Year by year predicted (circles) and observed (triangles) arrhythmic risk stratified by disease subtype: right dominant ACM (blue); bi-ventricular ACM (yellow); left dominant ACM (red). A clear divergence from model rates and a higher observed risk of events can be observed in the non-classical form cohorts.

Invasive Predictors

Invasive predictors non included in the *Cadrin-Tourigny model* were assessed; inducibility at PES and presence of Late Fragmented Potentials at EAM resulted strongly ($p<0.01$) associated with VAs in both

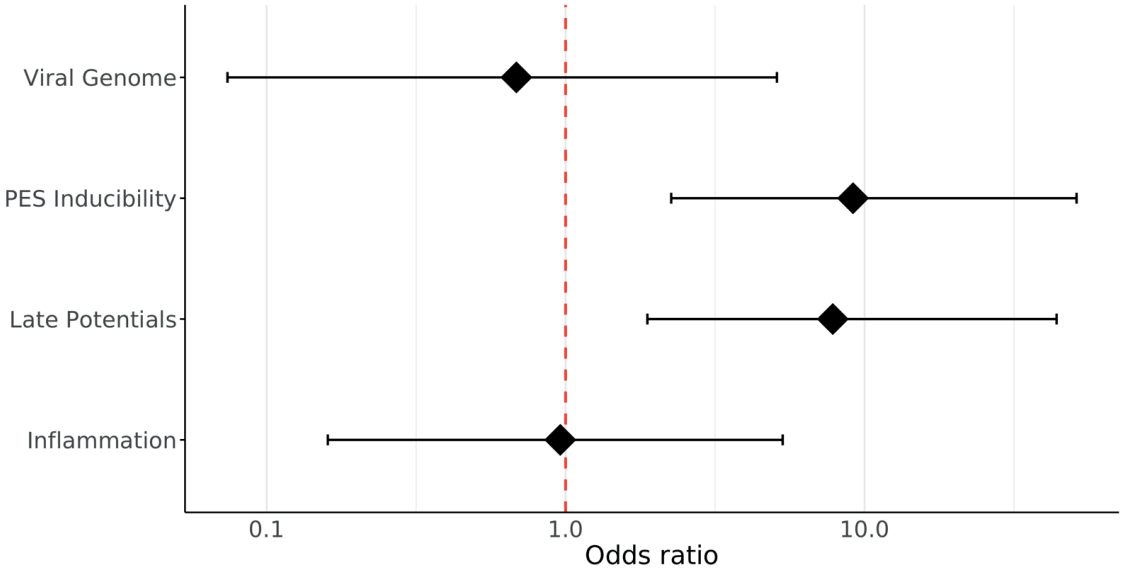
the whole cohort and sub-cohort; a sub-analysis assessing inducibility impact on the model goodness-to-fit in the overall and sub-cohorts has been reported in *Supplementary Appendix3*. Inflammatory and molecular pathology findings on EMB did not statistically correlate with VAs (Inflammation p=0.96; Viral Genome p=0.76). OR per specific analyzed predictor have been reported in **Figure6.**

Figure 5



R-ACM outcomes associated with different ICD implantation strategies are reported; from left to right: complete cohort implantation; increasing 5yr risk from Caudrin-Tourigni model indicated thresholds for implantation; no patient implantation; ITFC based implantation. Red represents ICD implantation occurrence (solid: implant; faded: no implant), blue represents ventricular arrhythmias during follow up (solid: presence; faded: absence); circles represent the net-clinical benefit (ICD:VA), e.g. the number of ICD placement needed to protect one patients from VAs. ICD: implantable cardioverter-defibrillator; ITFC: International Task Force Consensus; VA: ventricular arrhythmia

Figure 6



Odds of association between different invasive parameters and ventricular arrhythmias during the follow up. Data are reported on a logarithmic scale.

DISCUSSION

Since the increase of genetic testing and of CMR availability in bedside clinical practice, reports of non-classical ACM forms with early left ventricle involvement have been increasingly described(1–5). While a late left ventricle involvement has been associated with a poor long term outcome and malignant arrhythmias in several studies(3,14), to the best of our knowledge, a long term outcome analysis of ACM patients with non-classical forms with early left ventricle involvement (LD-ACM or Bi-ACM forms) is still lacking.

The population enrolled is composed of all consecutive patients with an ACM diagnosis undergoing a non-invasive and invasive diagnostic work-up at our institution to assess and characterized disease phenotype. Our patient population is older if compared to similar ACM cohorts(7–9): this is probably due to the low percentage of family members enrolled in the study, usually diagnosed at a younger age

than that of probands. The latter have also been shown to be at a higher arrhythmic risk than relatives, due to their later diagnosis(7): this may explain the higher overall risk and the slightly lower 5yr/freedom-from-VAs (0.65% for the overall cohort) reported in this study, compared to other third level center experience(7–9,11).

The invasive evaluation performed in this study, although requiring additional resources and potentially slightly increasing patient risk (literature reported peri-procedural risk for PES and EAM in high volume centers resulting indeed exceedingly low), allowed a deep characterization of the cohort. Around a third of patients resulted inducible at a PES, while fragmented late potentials were retrieved in about 40% of patients. Both predictors were associated in several studies to arrhythmic events during follow up (15–18), and resulted strongly associated to VAs in our study cohort as well (OR 9.15 and 7.83, respectively). Although currently not included in the ITFC consensus algorithm, they may represent an additional tool during ICD evaluation, especially in those patient falling in the intermediate ITFC risk category. EAM resulted also effective in guiding EMB for disease diagnosis: samples of ACM pathological fibro-fatty infiltration were retrieved in 55% and 84% of the samples performed before and after the introduction of the EAM-guided protocol, respectively, showing a clear improvement in EMB performance when EAM guided (13). Inflammatory cells and cardiotropic virus presence in myocardial samples from ACM patients has been described in previous studies(19–21). Although the role of inflammation and viral infection in disease pathogenesis remains unclear, no association with VA events in the study cohort during follow up was found,. Therefore, EMB seems to remain a powerful diagnostic, but not prognostic tool in the physician armamentarium during ACM assessment.

Thirty-three Non Classical ACM form patients were included into the study. In a recent genetic literature review, early left ventricular involvement was more commonly reported in patients with a DSP genetic mutation(22): *DSP* mutations were frequently found in the non-classical ACM sub-cohort in this study, resulting to be the most disease causing gene within the LD-ACM sub-cohort. Typically, LD-ACM

patients are older than R-ACM or Bi-ACM patients at disease diagnosis: an early left ventricle involvement alone is not a "classical" feature of the disease and a non-classical ACM diagnosis may not be high on list of differentials, leading to delay in referral and diagnosis. Many patients highly suspicious for LD-ACM may also not qualify for ITFC diagnosis, due to the lacking of specific cut-offs for left ventricle involvement in the 2010 consensus(12); in these patients, disease diagnosis is often reached with genetic analysis and EMB. Non Classical ACM forms with early left ventricular involvement at disease presentation were strongly associated with a worse long term outcome (p=0.04), as shown in Figure1a; in the inducibility sub-analysis reported in the Appendix4-Online Materials, long term outcome of Non Classical ACM was found comparable with the one of classical ACM patients resulting inducible at PES; thus, early left ventricle involvement appears to be a significant clinical parameter to be considered when assessing the long term arrhythmic risk of patient.

Cadrin-Tourigny et al Model External Analysis

VAs primary prevention with ICDs based upon risk stratification represents one of disease management cornerstones in ACM patients(6); however, until very recent days, accurate arrhythmic risk prediction models for primary VA prevention and ICD placement guidance were lacking. Following the ITFC model in fact, an extensive ICD primary protection coverage was obtained but at the expense of a high ICD-placed-per-treated-VA ratio. Following large implantable cardiac devices registry analyses quantifying the economic burden and the device-related risks carried by ICD placement(23–25), improvement in patient selection strategies for a better targeted ICD therapy were advocated. Recently, *Cadrin-Tourigny et al* developed and presented a novel arrhythmic risk prediction model from a multi-centered ACM registry(11), for which an online calculator has been made available at https://arvcrisk.com. This study sought to provide external validation to the algorithm and to assess its reliability in non-classical forms.

In our cohort, 82 patients resulted appropriate for algorithm use and their yearly risk from 1- to 5- years was calculated; observed VA rates during follow up resulted comparable in the overall population, even if a qualitative positive delta was observable at Figure2. When stratifying per ACM subtypes (Figure 3), several conclusions may be postulated:

1. *The Cadrin-Tourigny et al* model appeared perfectly adequate in predicting long-term patient risk in R-ACM patients; the best model predicted risk / observed VAs correlation was found at 4 and 5 years of follow up. *Cadrin-Tourigny et al* proposed the use of predicted 5-year risk as a decisional parameter for primary prevention ICD implant: from this external cohort long term data the model 5 year risk predictivity appears to be very reliable, and therefore a suitable parameter for decision making;
2. Non classical form arrhythmic risk appears to be under-predicted by the *Cadrin-Tourigny et al model*. Several reasons may be offered as explanation: a) non-classical forms appear at higher baseline arrhythmic risk than classical, causing divergence from algorithm predictions; b) In non-classical forms ventricle function contribution in VA risk has not been properly assessed yet and a RVEF impairment may not be present at all, eluding therefore model predictions; c) Non-classical forms are usually diagnosed at a later age but this is due to a non-classical disease presentation more than to a benign disease course;
3. At a net-benefit analysis, the *Cadrin-Tourigny et al* model for ICD placement resulted superior to classical forms in this external validation cohort for 5-year predicted risk thresholds ranging from 15 to 20%; using 15% as a threshold would have led to a better population protection with the same number of ICDs placed, while a 20% threshold would have offered the same ITFC VA protection but at a lower implanted ICD cost (Figure4; Table S3b–Online Supplement). A prospective validation trial is required to definitively assess model superiority;

4. PES inducibility was proven associated to VAs predicted risk but not included in the final *Cadrin-Tourigny et al* model; a higher discrepancy between model predicted-risk and observed VA rates was observed in patients resulting PES inducible and resulted clinically most significant (36% of underestimation) in the non-classical form sub-cohort.

Study Limitations

The study cohort has been extracted from the single-center registry of a tertiary institution; a referral could not be ruled out and the rate of VA events may be overestimated. These results apply to an invasively-studied cohort mostly consisting of ACM probands with a certain ACM diagnosis and could not be generalized to the whole spectrum of patients with ACM. The study was also a retrospective descriptive and a first external validation study for a novel model: although showing great benefit potentials, further multicenter prospective studies with larger samples are needed before a routine use of the *Caudrin-Tourigny et al* model could be introduced in general practice in a community setting.

CONCLUSIONS

Non classical ACM forms with early left ventricle involvement are a not-so-uncommon clinical entity and appear to be more prone to VA events than classical right dominant ACM forms. The novel *Caudrin-Tourigny et al* algorithm appeared very effective in predicting long term arrhythmic risk and in guiding ICD placement in this external validation cohort of probands with the classical ACM form requiring invasive investigation. In the non-classical forms, the algorithm appears to underestimate clinical risk; an integration with invasive assessment techniques, such as PES and EAM, should be considered in those forms presenting with an early left ventricle involvement.

SOURCE OF FUNDING:

Logistical support was given by the Centro Cardiologico Monzino IRCCS institution. C.B. is supported by the Registry for Cardio-cerebro-vascular Pathology, Veneto Region, Italy and PRIN MIUR Project and Ministry of Health Target Project. Rome, Italy.

DISCLOSURES:

C.T. received modest honoraria from St. Jude Medical and Abbott and serves as member of the advisory board for Medtronic, Inc and Boston Scientific Corp; L.D.B is a consultant for Biosense-Webster, Boston-Scientific and St-Jude Medical and has received modest honoraria from Medtronic, Atricure, EPiEP. A.N. is a consultant for Boston-Scientific, Biosense-Webster, St.-Jude Medical, Biotronik and Medtronic and Biotronik. Remaining authors declare no pertinent conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary Appendix1 - CMR protocol

CMR studies were performed with a 1.5-T unit (Discovery MR450, GE-Healthcare, Milwaukee, MN). All studies were carried out using dedicated cardiac software, phased-array surface receiver coils, and electrocardiogram triggering. Breath-hold steady-state free-precession cine imaging was performed in vertical and horizontal long-axis and in short-axis orientations. A stack of short-axis slices encompassing both ventricles from base to apex was used for biventricular volumes, mass and systolic function assessment. In addition, for ruling out ACM, a set of axial long-axis views from diaphragm to the right ventricular outflow tract was acquired. The following acquisition parameters were applied: 30 phases, 10-25 views per segment, NEX 1, FOV 40 cm, a matrix of 224 x 224, a 60° flip angle, TR 3.6-4.2 and TE = TR/2. For detecting fat infiltration, the FSE/STIR method was used (17). Conventional breath hold T1 weighted fast spin echo images were acquired in the same short-axis views (8-mm slice thickness, no gap) and long-axis views with the following parameters: for FSE NEX 1, FOV 40 cm, matrix of 256x256, TR 1 RR interval and TE minimum (range 4.5-7.8 ms). A breath-hold short-TI inversion-recovery (STIR) spin-echo pulse sequence was used in the same short-axis and long-axis views with the following parameters: NEX 1, FOV 400 mm, TR 2 R-R intervals, TE 60 ms, TI 150 ms, matrix 256 9 256 and slice thickness 8 mm. A contrast-enhanced breath-hold segmented T1-weighted inversion-recovery gradient-echo sequence was used for myocardial fibrosis detection using the LGE technique. LGE-imaging was performed 10-20 minutes after administration of an intravenous bolus of 0.1 mmol/kg gadolinium-BOPTA (Multihance, Bracco, Milan, Italy). Inversion time was individually adapted to null the signal of remote myocardium (usual range 220-300 ms). The following parameters were used: FOV: 380-420 mm, TR/TE 4.6/1.3 ms, α 20°, matrix 256x192, ST 8 mm and no inter-slice gap.

CMR analysis

All exams were centrally analyzed at our center. CMR datasets were transferred to a dedicated workstation and analyzed with a cardiac software (Report Card 4.0, GE-Healthcare, Milwaukee, WI) by two expert readers blinded to patient clinical history and data. For any disagreement on data analysis between the two readers, consensus agreement was achieved involving a third expert reader. On the stack of cine short-axis images, epicardial and endocardial contours were outlined by manual contouring and the papillary muscles were included in LV myocardial mass. Left

ventricular volumes, stroke volume and ejection fraction were also quantified using the stack of cine short-axis images. Left ventricular volume, stroke volume and mass were normalized to body surface area. Right ventricle abnormalities such as right ventricle dilation, reduction of right ventricle ejection fraction, abnormalities of free wall kinesis and right ventricle LGE were assessed.

Supplementary Appendix2 – EAM cut-offs

When performing EAM and EAM-guided EMB, the following cut-offs were used to define pathological and healthy myocardial areas:

- RV: bipolar potentials abnormal when < 1,5 mV; unipolar potentials abnormal when < 5.5 mV;
- LV: bipolar potentials abnormal when < 1,5 mV; unipolar potentials abnormal when < 8 mV.

Supplementary Table S1 Patients Without Sustained VAs and/or TCA at Disease Presentation Baseline Characteristics

	Overall	R-ACM	BI-ACM	LD-ACM
Total	82 (100)	51 (62.2)	14 (17.1)	17 (20.7)
Demographics Characteristics				
Age at Diagnosis, years (mean±s.d.)	41.3±14.4	39.±13.8	39.4±11.6	48.8±16.3
Male, n (%)	60 (73.1)	37 (72.6)	10 (71.4)	13 (76.5)
Proband, n (%)	71 (86.6)	42 (82.3)	12 (85.7)	17 (100)
Recent Cardiac Syncope, n (%)	6 (7.3)	3 (5.8)	2 (14.3)	1 (5.9)
Genetic Analysis, n (%)	51 (62.0)	31 (60.8)	8 (57.1)	12 (70.6)
History of NSVT, n (%)	28 (34.2)	14 (27.5)	5 (35.7)	9 (52.9)
Baseline and Holter ECG				
TWI in ≥3 precordial leads, n (%)	39 (47.6)	25 (49.0)	8 (57.1)	6 (35.3)
TWI in ≥2 inferior leads, n (%)	20 (24.4)	8 (15.7)	4 (28.6)	8 (47.1)
N of inverted T waves, n (mean±s.d)	2.6±1.7	2.4±1.6	2.8±2.2	2.9±1.6
24hPVC burden, n [R.I.Q]	850 [305-2257]	1000 [310–2522]	700 [478 – 1501]	669 [80–1727]
CMR Imaging				
RVEF, % (mean±s.d.)	47.4±9.3	46.0±7.5	40.8±8.7	56.8±7.7
LVEF, % (mean±s.d.)	50.7±10.9	54.6±9.0	46.3±11.4	42.7±10.4
Invasive Evaluation Data				
PES inducibility, n (%) [*2]	27 (33.7)	16 (32.7)	3 (21.4)	8 (42.1)
Low EAM Unipolar Potentials, n (%) [*2]	52 (63.0)	31 (63.3)	11 (78.6)	10 (58.8)
Low EAM Bipolar Potentials, n (%) [*2]	46 (57.5)	26 (53.0)	10 (71.4)	10 (58.8)
Late Fragmented EAM Potentials, n (%) [*2]	29 (36.2)	15 (30.6)	7 (50.0)	7 (41.2)
EMB, n (%)	49 (59.8)	29 (56.9)	8 (57.1)	12 (70.6)
Treatment at Baseline				
ICD, n (%)	54 (65.9)	29 (56.9)	8 (57.1)	12 (70.6)
AADs, n (%)	32 (44.4)	15 (36.6)	7 (50.0)	10 (58.8)
B-Blockers, n (%)	42 (58.3)	20 (48.8)	8 (57.1)	14 (82.3)

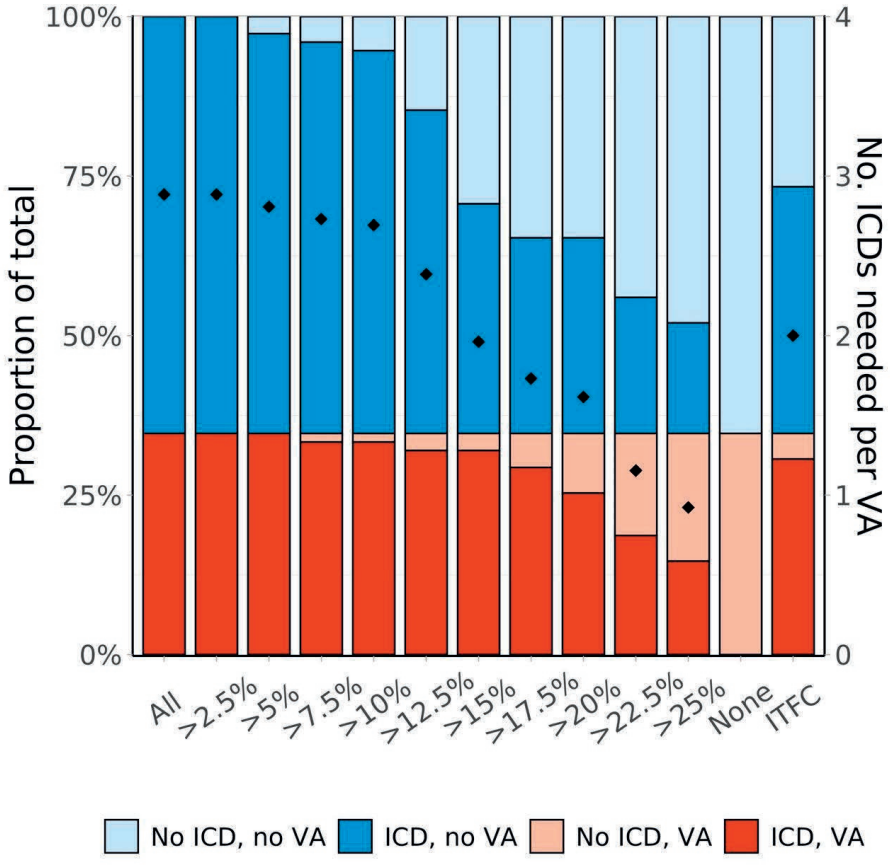
Supplementary Table S2 – Risk Analysis Data

	Overall	R-ACM	BI-ACM	LD-ACM
Total	82 (100)	51 (67.3)	14 (13.9)	17 (18.8)
Risk as per CT et al algorithm				
1-year estimated risk, % (mean±s.d)	9.63 ± 8.1	9.8±8.8	11.3±5.7	7.8±7.5
2-year estimated risk, % (mean±s.d)	14.9 ± 11.3	15.2±12.1	17.5±8.5	12.0±10.9
3-year estimated risk, % (mean±s.d)	18.6 ± 13.0	18.2±13.6	22.1±9.21	16.2±14.2
4-year estimated risk, % (mean±s.d)	20.3 ±13.7	19.7±14.3	24.7±9.6	17.9±15.0
5-year estimated risk, % (mean±s.d)	24.3 ± 15.3	23.6±15.7	29.7±10.9	21.4±17.1
Recurrence Rate (RR)				
1-year RR, %	13.9	8.33	21.4	23.5
2-year RR, %	16.7	10.6	28.6	23.5
3-year RR, %	23.9	15.2	38.5	41.7
4-year RR, %	31.9	20.0	58.3	50.0
5-year RR, %	34.8	24.4	58.3	50.0
Predicted/Observed % Difference, value [95% C.I.]	+2.7 [- 0.8; +6.2]	+1.6 [-10,2; +7.1]	-20.0 [-1.1;-38.9]	-22.6 [-7.8; -37.5]

Supplementary Table S3 – Overall Model Clinical Impact

CT derived implant threshold	All	>5%	>10%	>12.5%	>15%	>17.5%	>20%	>22.5%	ITFC
VA, ICD	26	26	25	24	24	22	19	14	23
	(34.7%)	(34.7%)	(33.3%)	(32.0%)	(32.0%)	(29.3%)	(25.3%)	(18.6%)	(30.6%)
VA, no ICD	0	0	1	2	2	4	7	12	3
	(0%)	(0%)	(1.3%)	(2.6%)	(2.6%)	(5.3%)	(9.3%)	(16.0%)	(4.0%)
No VA, ICD	49	47	45	38	27	23	23	16	29
	(65.3%)	(62.6%)	(60.0%)	(50.6%)	(36.0%)	(30.6%)	(30.6%)	(21.3%)	(38.6%)
No VA, no ICD	0	2	4	11	22	26	26	33	20
	(0%)	(2.6%)	(5.3%)	(14.7%)	(29.3%)	(34.6%)	(34.6%)	(44.0%)	(26.7%)
ICD total	75	73	70	62	51	45	42	30	52
	(100%)	(97.3%)	(93.3%)	(82.7%)	(68.0%)	(60.0%)	(56.0%)	(40.0%)	(69.3%)
Net Benefit (ICD:VA rate)	2.88	2.81	2.69	2.38	1.96	1.73	1.62	1.15	2.00

Related Supplementary Figure S3 – Overall Model Clinical Impact



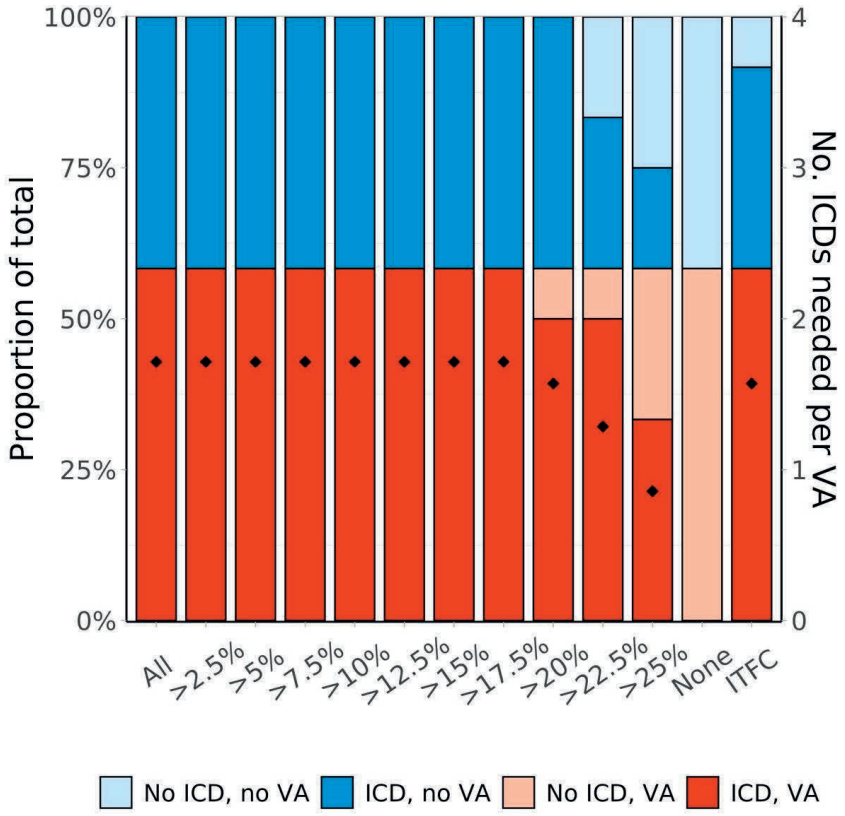
Supplementary Table S3a – R-ACM Model Clinical Impact

CT derived threshold	All	>5%	>10%	>12.5%	>15%	>17.5%	>20%	>22.5%	ITFC
VA, ICD	13	13	13	13	13	12	10	5	10
	(25.5%)	(25.5%)	(25.5%)	(25.5%)	(25.5%)	(23.5%)	(19.6%)	(9.8%)	(19.6%)
VA, no ICD	0	0	0	0	0	1	3	8	3
	(0%)	(0%)	(0%)	(0%)	(0%)	(1.9%)	(5.8%)	(15.9%)	(5.8%)
No VA, ICD	38	37	35	29	18	17	17	12	21
	(74.5%)	(72.5%)	(68.6%)	(56.9%)	(35.3%)	(33.3%)	(33.3%)	(23.5%)	(41.1%)
No VA, no ICD	0	1	3	9	20	21	21	26	17
	(0%)	(1.9%)	(5.8%)	(17.6%)	(39.2%)	(41.1%)	(41.1%)	(51.0%)	(33.3%)
ICD total	51	50	48	42	31	29	27	17	31
	(100%)	(98.1%)	(94.1%)	(82.4%)	(60.7%)	(56.9%)	(52.9%)	(33.3%)	(60.8%)
Net Benefit (ICD:VA rate)	3.92	3.85	3.69	3.23	2.38	2.23	2.08	1.31	2.38

Supplementary Table S3b – Bi-ACM Model Clinical Impact

CT derived threshold	All	>5%	>10%	>12.5%	>15%	>17.5%	>20%	>22.5%	ITFC
VA, ICD	7	7	7	7	7	7	6	6	7
	(58.3%)	(58.3%)	(58.3%)	(58.3%)	(58.3%)	(58.3%)	(50.0%)	(50.0%)	(58.3%)
VA, no ICD	0	0	0	0	0	0	1	1	0
	(0%)	(0%)	(0%)	(0%)	(0%)	(0%)	(8.3%)	(8.3%)	(0%)
No VA, ICD	5	5	5	5	5	5	5	3	4
	(41.7%)	(41.7%)	(41.7%)	(41.7%)	(41.7%)	(41.7%)	(41.7%)	(25.0%)	(33.3%)
No VA, no ICD	0	0	0	0	0	0	0	2	1
	(0%)	(0%)	(0%)	(0%)	(0%)	(0%)	(0%)	(16.6%)	(8.3%)
ICD total	12	12	12	12	12	12	11	9	11
	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(91.7%)	(75.0%)	(91.7%)
Net Benefit (ICD:VA rate)	1.71	1.71	1.71	1.71	1.71	1.71	1.57	1.29	1.57

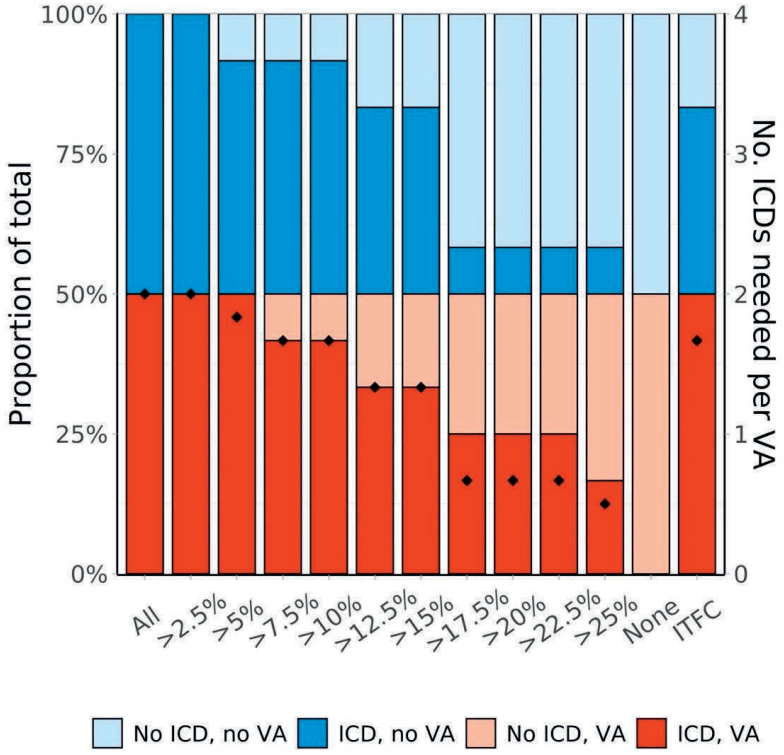
Supplementary Related Figure S3b– Bi-ACM Model Clinical Impact



Supplementary Table S3c – LD-ACM Model Clinical Impact

CT derived implant threshold	All	>5%	>10%	>12.5%	>15%	>17.5%	>20%	>22.5%	ITFC
VA, ICD	6	6	5	4	4	3	3	3	6
	(50.0%)	(50.0%)	(41.6%)	(33.3%)	(33.3%)	(25.0%)	(25.0%)	(25.0%)	(50.0%)
VA, no ICD	0	0	1	2	2	3	3	3	0
	(0%)	(0%)	(8.3%)	(16.7%)	(16.7%)	(25.0%)	(25.0%)	(25.0%)	(0%)
No VA, ICD	6	5	5	4	4	1	1	1	4
	(50.0%)	(41.6%)	(41.6%)	(33.3%)	(33.3%)	(8.3%)	(8.3%)	(8.3%)	(33.3%)
No VA, no ICD	0	1	1	2	2	5	5	5	2
	(0%)	(8.3%)	(8.3%)	(16.7%)	(16.7%)	(41.6%)	(41.6%)	(41.6%)	(16.7%)
ICD total	12	11	10	8	8	4	4	4	10
	(100%)	(91.6%)	(83.3%)	(66.7%)	(66.7%)	(33.3%)	(33.3%)	(33.3%)	(83.3%)
Net Benefit (ICD:VA rate)	2.0	1.83	1.67	1.33	1.33	0.67	0.67	0.67	1.67

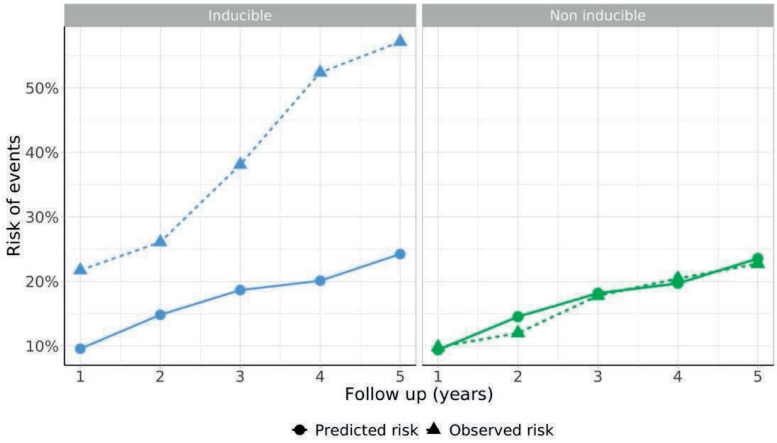
Supplementary Related Figure S3c – LDACM Model Clinical Impact



Supplementary Appendix 3 – Inducibility Analisis

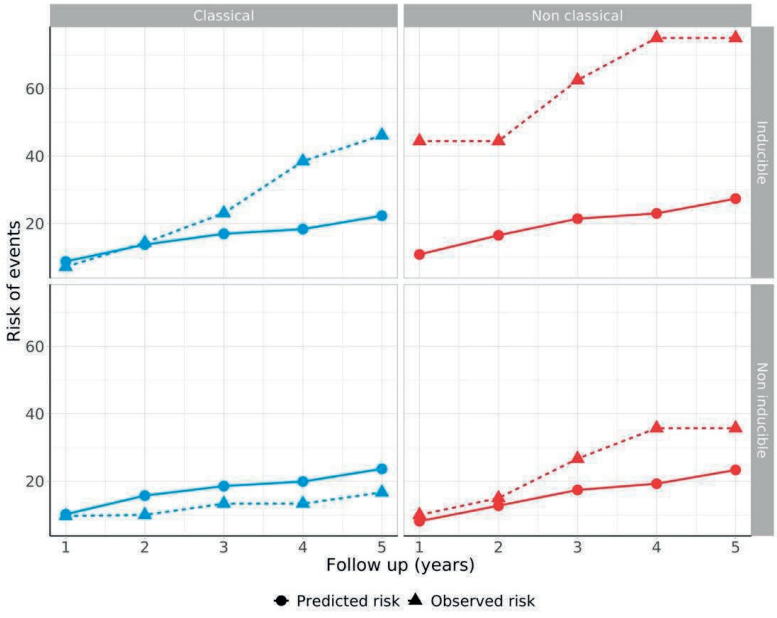
Inducibility at a PES has been strongly associated with VAs during follow up in several studies and it was proven a significant prognostic factor even in the recent study from *Cadrin-Tourigny et al*, although it was not included in the final algorithm (1,2). There is no clear consensus on the need for routine PES testing in ACM patient: it represents an expansive and invasive (although very low risk), second level test whose results however may help assessing intermediate risk patient evaluation and ICD placement decision. In the study cohort, 35 (35.4%) patients resulted inducible at PES; the novel risk predicting model underestimated the risk of the overall inducible population , while almost perfectly predicting the risk of non-inducible patients, as reported in Supplementary Figure S5. When stratifying by classical and non-classical form the analysis (Supplementary Figure S6), inducibility was shown to be a drifting factor from algorithm predictions; inducibility impact on predicted and observed rates resulted maximum in the non-classical forms, where it was associated with 36.2% (95% C.I 24.1 – 48.2 %; $p < 0.001$) mean difference.

Supplementary Figure S5



Predicted (circles) and Observed (triangles) arrhythmic risk stratified by electrical inducibility in the overall cohort over follow up: a greater difference between predicted and observed risk is observed in the inducible sub-cohort (blue) than in the non-inducible one (green).

Supplementary Figure S6



Predicted (circles) and Observed (triangles) arrhythmic risk stratified by electrical inducibility and disease phenotype (Non Classical Forms: red; Classical Forms: blue) in the overall cohort over follow u

SUPPLEMENTARY REFERENCES

1. Saguner AM, Medeiros-Domingo A, Schwyzer MA, On C-J, Haegeli LM, Wolber T, et al. Usefulness of Inducible Ventricular Tachycardia to Predict Long-Term Adverse Outcomes in Arrhythmogenic Right Ventricular Cardiomyopathy. The American Journal of Cardiology. 2013 Jan;111(2):250–7.
2. Cadrin-Tourigny J, Bosman LP, Nozza A, Wang W, Tadros R, Bhonsale A, et al. A new prediction model for ventricular arrhythmias in arrhythmogenic right ventricular cardiomyopathy. European Heart Journal [Internet]. 2019 Mar 27 [cited 2019 Apr 26]; Available from: https://academic.oup.com/eurheartj/advance-article/doi/10.1093/eurheartj/ehz103/5419784

CHAPTER 4

NOVEL RISK CALCULATOR PERFORMANCE IN ATHLETES WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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Heart Rhythm. *2020 Aug;17(8):1251-1259. doi: 10.1016/j.hrthm.2020.03.007.*PMID: 32200046

ABSTRACT:

Background: disease progression and ventricular arrhythmias (VAs) in arrhythmogenic right ventricular cardiomyopathy (ARVC) are correlated with physical exercise, with clinical de-training and competitive sport practice avoidance being suggested in ARVC patients. A recent algorithm assessing primary arrhythmic risk in ARVC patients has been developed by Cadrin-Tourigny et al . Data regarding its transferability in athletes are lacking.

Objective: to assess reliability of the Cadrin-Tourigny risk prediction algorithm in a cohort of athletes with ARVC; to describe impact of clinical detraining on disease progression.

Methods: all athletes undergoing clinical de-training after ARVC diagnosis at our institution were enrolled. Baseline and during follow up clinical characteristics and VAs events during follow up were collected. The Cadrin-Tourigny algorithm was used to calculate the a-priori predicted VA risk, which was compared with the observed outcomes.

Results: twenty-five athletes (36.1±14.0 years, 80% male) with definite ARVC, undergoing clinical detraining, were enrolled. Over a median follow up of 5.3 [3.2–6.6] years, a reduction in the PVCs burden (p=0.001) was assessed and 10 (40%) VA events were recorded. The a-priori algorithm predicted risk appeared to fit with the observed cohort arrhythmic risk (mean observed-predicted risk difference over 5 years: -0.85% [-4.8–3.1]; p=0.85). At one-year follow up, 11 (44%) pts improved their stress-ECG response, while no significant changes in RVEF were observed.

Conclusion: clinical de-training is associated with PVCs burden reduction in athletes with ARVC. The novel risk prediction algorithm does not appear to need any correction for its application in ARVC athletes.

BACKGROUND:

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is an inherited cardiomyopathy characterized by predominant but not exclusive right ventricular myocardial fibro-fatty replacement[1–3]. ARVC is usually characterized by electrical and morphological RV alterations that have notoriously associated this disease with an important increase in sudden cardiac death (SCD) risk due to malignant ventricular arrhythmias[1,3]. Implantable cardioverter defibrillators (ICDs) represent a viable option to deal with SCD risk in this patient population[4], but until recently, appropriate tools for an adequate individual primary arrhythmic risk stratification were scarce[5–8]. Recently, a novel algorithm for primary prevention ICD placement has been developed from a large sample size international registry[9]. Its performance appears to be superior to the International Task Force (ITF) consensus statement criteria from 2015, possibly leading to a better patient tailored therapy and increasing net clinical benefit[9].
A pivotal role in ARVC disease progression and malignant ventricular arrhythmia (VA) genesis is held by engagement in sports, with several studies assessing this correlation[10–14]; physical exercise greatly increases arrhythmic risk in these patients and an appropriate de-training and sport practice reduction after disease diagnosis reducing the long term risk of VAs[15]. No specific adaptation for the individual level of physical exercise is present in the current *Cadrin-Tourigny et al* algorithm (www.arvcrisk.com); data for validation of this algorithm in athletes is currently lacking and its transferability to an external high-intensity athlete cohort with ARVC yet to be tested.This study therefore aims to validate the *Cadrin-Tourigny et al* algorithm and report on disease progression in a well characterized high intensity athlete cohort of patients with ARVC.

METHODS:

All consecutive athletes with a definitive ARVC diagnosis evaluated at Arrhythmology and sport medicine unit of IRCCS Centro Cardiologico Monzino were enrolled in the study. This analysis was

approved by the local ethic review board according to center legislation and complies with the Declaration of Helsinki.

Cohort definition:

ARVC diagnosis was postulated by a dedicated heart team composed of cardiac radiologists, electrophysiologists, and cardiac pathologists, in accordance to the 2010 Revised Task Force Criteria[16]. A patient was classified as an athlete after a complete sport cardiology evaluation by dedicated physicians on the basis of training regime (>6h/week of sports with a moderate-to-intense dynamic components (at least 6 METs in intensity); affiliation to an Italian or Internationally recognized sport federation; regular sportive competitions over the year; sport history without significant breaks/change in training patterns/load over the last three years)[15,17]. Endurance sports were defined as those requiring sustained efforts at >70% VO_2 max (such as cycling, swimming, rowing), while mixed sported were defined as those in which a mix of skill-based and aerobic/anaerobic exercise is required (such as football, basketball, volleyball)[18]. All patients regularly practiced their discipline up until disease diagnosis or had their sport eligibility suspended immediately before referral to our center.

Patient in Hospital Evaluation:

After referral to our centers through the outpatient clinic or during hospital in-stay in case of direct admission, all enrolled athletes routinely underwent 12-lead baseline ECG, 24-hour Holter ECG Monitoring, complete blood panel, ECG-stress test, ARVC dedicated cardiac ultrasound, and cardiac magnetic resonance (CMR) analysis. As part of the arrhythmic risk assessment, a baseline programmed electrical stimulation (PES) was performed routinely. Three dimensional (3D) endo-cavitary electro-anatomical mapping (EAM), percutaneous EAM guided endo-myocardial biopsy (EMB), and genetic testing for diagnostic purposes were performed according to physicians' expertise and indication. SCD risk stratification analyses were performed in all patients as per the ITFC Statement for ARVC treatment[4], and ICD placement performed accordingly.

Evaluation of Arrhythmic Events Evaluation and Patient Follow Up:

At disease diagnosis, the 24-hour premature ventricular complex (PVC) burden and all complex arrhythmic events [Non-sustained ventricular tachycardia (NSVT); sustained ventricular tachycardia (SVT); ventricular fibrillation/flutter (VF)] at baseline and in patient history were recorded, as well as the reason for first referral to our center. At disease diagnosis, patients' sport eligibility status was immediately suspended; patients were instructed to start a de-training period highly reducing their training regimen and abstaining perpetually from a competitive sport practice as per international sport medicine guidelines[19,20].

Patients were followed up for disease progression at 6 months after hospital discharge and subsequently every 12-months, or immediately after the occurrence of an arrhythmic event and/or an emergency room access. An Holter-ECG was required at 6-months, then surveillance protocol required a minimum of one 24-h Holter every 24 months but individualized monitoring protocols were scheduled accorded to physician expertise and patients availability. In case of patients with an ICD, an ICD interrogation was performed by dedicated personnel every 6-8 months and a summary check was performed contextually every follow up visit. Stress testing ECG, cardiac ultrasound, and CMR follow up surveillance schemes were not mandated per protocol.

Study Outcomes:

The first sustained VA event after disease diagnosis was the primary outcome of the study; sustained VAs were defined as a composite of SCD, SVT, VF, or an appropriate ICD intervention on any of the previous arrhythmias.

For all patients complying to model requirements (as reported in the referred study and on the www.arvcrisk.com website)[9], the expected VA rate was calculated using the *Cadrin-Tourigny et al* predictive model. The yearly risk was calculated at disease diagnosis and using the values of the indicated

variables as measured at disease diagnosis. Observed sustained VA rate at long term follow up was calculated and compared to the algorithm predicted rate. Changes in 24-hour Holter ECG PVC number, stress ECG result, and RV ejection fraction (EF) modification pre and post de-training protocol were also collected and analyzed as secondary outcomes.

Statistical Analysis:

All statistical analyses were performed using R Project for statistical computing version 3.5. Continuous variables were expressed as mean±standard deviation or as median [interquartile range (IQR)], while categorical variables were expressed as counts (%); comparisons were performed using the independent sample t-test or the Mann-Whitney U test, as appropriate. Kaplan-Meier analysis with log-rank test were used for statistical inference on long term outcome data. Predicted and observed frequencies of sustained VAs were evaluated and compared with a multivariate linear regression model. To evaluate the effect of time over the total number of PVC and RVEF we fit a mixed model to the data in which time was considered the fixed effect and the subject as the random effect. The model ignores the missing data but generates an output with the same observation number as in the original dataset. The findings were considered statistically significant with a two-tailed p value <0.05.

RESULTS:

A total of 25 athletes with a definitive ARVC diagnosis were enrolled in this study, with males representing the cohort's majority (80%). The mean age at disease diagnosis was 36.1±14.0 years. All patients were probands, with 6 (24%) patients presenting a history of SCD in their family at an in-depth evaluation. Sport-wise, 14 (56%) patients practiced an aerobic-anaerobic mixed sport, while the remaining 11 (44%) were endurance athletes.

The most common reason for referral was the presence of abnormalities at sport eligibility assessment visit (n=18, 72%), followed by symptomatic VA index event (n=5, 20%), of which two required

emergency resuscitation maneuvers on the field due to sudden cardiac arrest. At first evaluation, the median number of PVCs in the 24/h Holter ECG was 1000 [300-3500]; seven (28%) patients had a history of documented NSVT, and 5 (20%) of documented sustained VAs; at CMR, mean RVEF and LVEF values resulted respectively 47.5±8.1 and 56.3±5.2. A genetic testing analysis was performed in 15 (60%) athletes of which 9 resulted positive for a pathogenic or likely pathogenic mutation. All patients underwent PES and a total of 9 (26%) patients had VAs induced in the electrophysiology lab; EAM and EAM-guided EMB were performed in 10 (40%) patients. After disease diagnosis and appropriate arrhythmic risk stratification, a total of 10 (40.0%) ICDs implantation (n=4 subcutaneous ICDs; n=5 transvenous dual-chamber ICDs; n=1 transvenous single chamber ICD) were performed; an additional S-ICD placement was deemed necessary but it was refused by the patient. A list of the study population characteristics has been reported in **Table1.**

Table 1

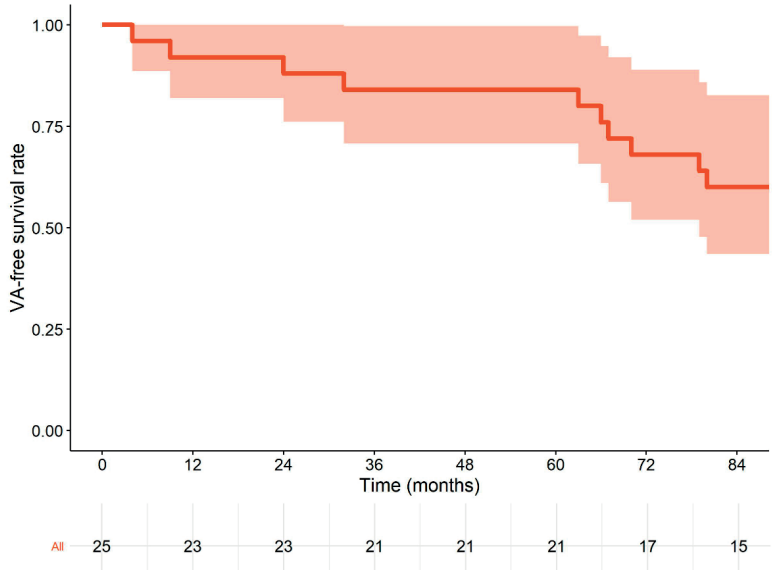
Patient Cohort Baseline Characteristics (n=25)	
Demographics Characteristics:	
Age at Diagnosis, years (mean±s.d)	36.1±14.0
Male, n (%)	20 (80.0)
Training load, hours (mean±s.d)	7.4±1.3
Sport Practice:	
Mixed Sports	14 (56.0)
Volley, n (%)	3 (12.0)
Football, n (%)	7 (28.0)
Basket, n (%)	4 (16.0)
Endurance Sports	11 (44.0%)
Cycling, n (%)	5 (20.0)
Endurance Running, n (%)	4 (16.0)
Triathlon, n (%)	1 (4.0)
Rowing, n (%)	1 (4.0)
Reason For Referral:	
Abnormalities at Sport Eligibility Visit	18 (72%)
ECG static abnormalities, n (%)	5 (20.0)
PVCs, n (%)	8 (32.0)
NSVT, n (%)	5 (20.0)
Complex VAs during PA, n (%)	5 (20%)
SVT, n (%)	3 (12.0)
VF with SCD, n (%)	2 (8.0)
Unexplained Loss Of Consciousness, n (%)	2 (8%)
Stress Test ECG:	
Negative, n (%)	6 (24.0)

PVCs – suppressed by PA, n (%)	10 (40.0)
PVCs – unsuppressed by PA, n (%)	5 (20.0)
NSVTs, n (%)	3 (12.0)
SVTs, n (%)	1 (4.0)
CMR Imaging	
RVEF, % (mean±s.d.)	47.5±8.1
LVEF, % (mean±s.d.)	56.3±5.2
Interventions:	
PES inducibility, n (%)	9 (36.0)
SVT, n (%)	7 (28.0)
VF, n (%)	2 (8.0)
ICD placement, n (%)	10 (40.0)
S-ICD, n (%)	4 (16.0)
TBC-ICD, n (%)	5 (20.0)
TSC-ICD, n (%)	1 (4.0)

Follow Up Arrhyhtmic Analysis

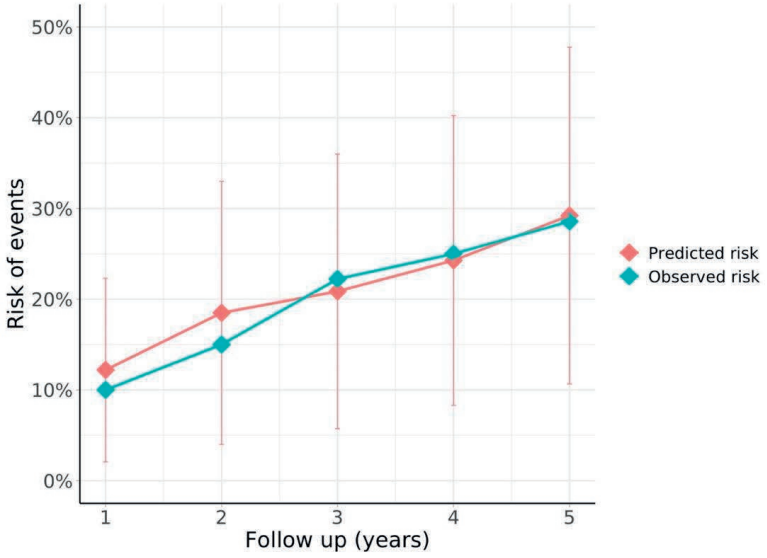
Over a median follow up time of 5.3 [3.2–6.6] years, a sustained VA event was documented in 10 (40.0%) athletes; of these episodes, 6 were recorded in previously ICD implanted patients. The cumulative freedom from VA events of the cohort has been reported in **Figure1** (Overall 5yr/freedom-from-VA rate 0.84 [0.71-0.92]). Within the cohort, 20 (80%) patients complied with the *Cadrin-Tourigny et al* algorithm requirements and their predicted risk was calculated. The clinical characteristics of this subpopulation are reported in *S-Table1*. The comparison between predicted and observed VA rates over time are reported in **Figure2**; events at follow up resulted well within the 95% CI of predicted rates, non-significantly differing from the algorithm predicted rate at any follow up time (mean difference observed-predicted rate over 5 years -0.85% [-4.80;+3.10]; p=0.85); the whole risk data analysis are reported in *S-Table2*.

Figure 1



Overall Freedom from VAs of the overall cohort.

Figure 2

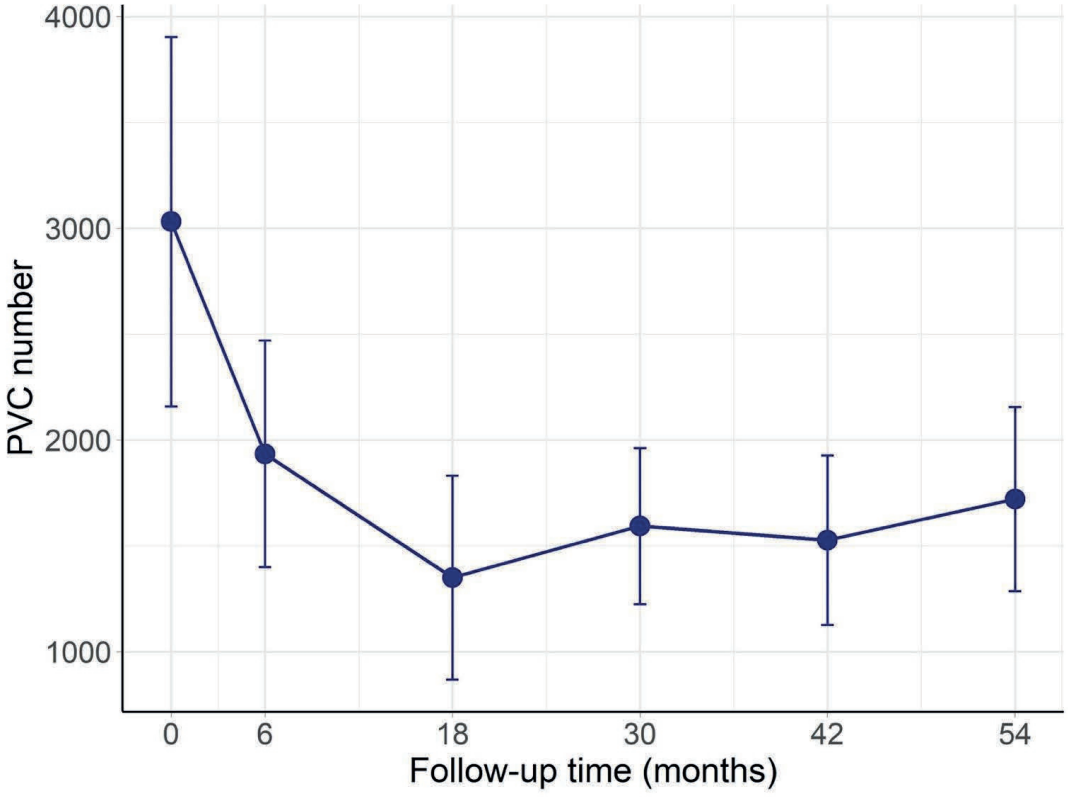


Comparison between observed and algorithm predicted event rates: the algorithm predicted risk showed almost perfect adherence to observed event rates over follow up. Time is considered from disease diagnosis.

Disease progression during follow up:

After disease diagnosis and the beginning of the de-training protocol, athletes characteristics were collected over time to monitor disease evolution. Over time, a statistically significant 24h/PVC count reduction was observed (p=0.001) (mean reduction over first 18-months: -1682±573, p=0.048; mean reduction from 18 to end-of-follow up 160±680, p=0.99) (**Figure3**).

Figure 3

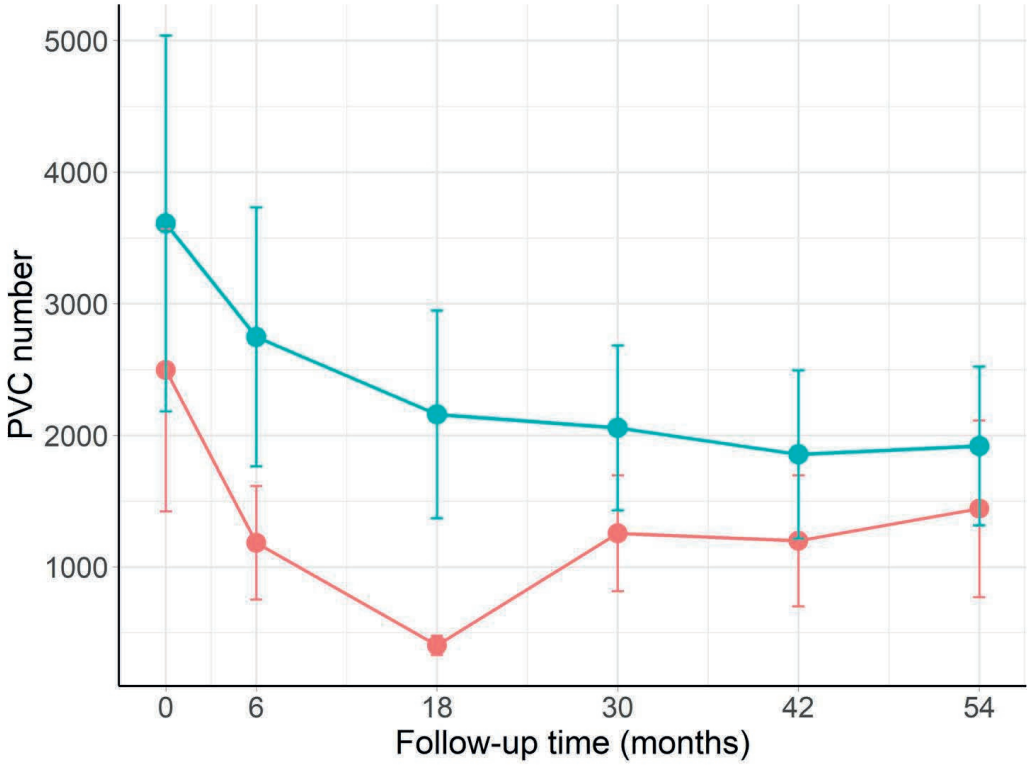


Mean PVC/24h number over follow up for the entire cohort: a clear reduction in PVC number can be already observed at six months from detraining. Time is considered from disease diagnosis.

A sub-analysis to assess beta-blocker influence on 24h/PVC count showed that the effect of clinical de-training on 24h/PVC count was not significantly influenced by the presence of beta-blocker (p=0.33)

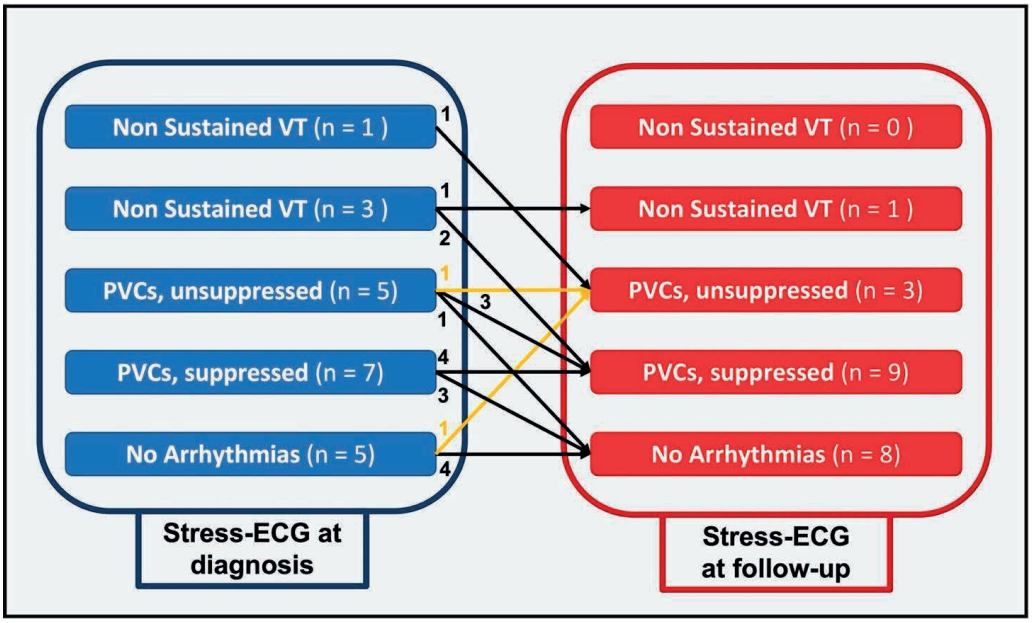
(Figure4). Over a median time of 8 [4–11] months, 21 (84%) patients repeated a stress-ECG. In these, a per-patient qualitative improvement in arrhythmic response at stress-testing was observed in 11 (52%) patients, 9 (43%) presented the same arrhythmic response and only 1 (5%; patient not following de-training protocol) presented a worsened arrhythmic stress-ECG response. Graphical representation of stress-ECG data over time is displayed in **Figure5**. A total of 18 (72%) patients repeated CMR exam after a median time of 12 [9–13] months, over which RVEF remained stable. (MRI RVEF mean change +0.11% [-2.31%;+2.54%], p=0.92; mean change excluding two patients not following de-training protocol +1.43% [-0.23;+3.11%], p=0.09) (**Figure6**).

Figure 4



Mean PVC/24h number over follow up when stratifying for beta-blockers (Teal line: patients on beta-blocker; red line: patients off beta-blocker). No significant difference in trend reduction between the two groups was observed. Time is considered from disease diagnosis.

Figure 5

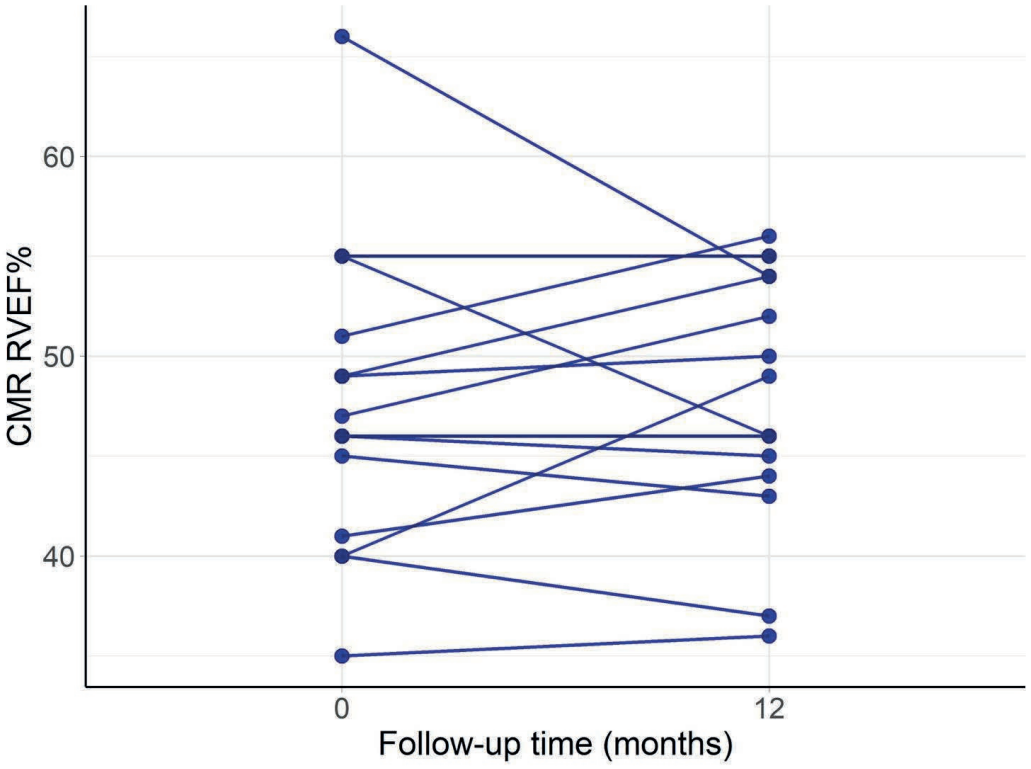


Changes in stress-ECG arrhythmic response over time

DISCUSSION:

ARVC represents the first cause of sudden death among young individuals and this risk has been described as five-fold increase in active athletes[10,21], specifically in those training with high intensity regimen and participating in endurance sport competitions[3,10]. Physical activity has been demonstrated increasing disease progression and overall arrhythmic risk in this patient population[11–14,22,23], with high-end strenuous exercise and endurance training being associated with the worst outcomes[24]. In light of this fact, the current sport medicine guidelines suggest complete abstention from competitive sport training in ARVC patients and a significant reduction even in leisure time sport practice time[19,20].

Figure 6



Changes in CMR RVEF over time

Current scientific consensus indicates ICD placement in high arrhythmic patient after arrhythmic risk assessment as the most effective treatment for ARVC patients[4]. However, a high rate of ICD implanted per VA treated as been reported in ARVC literature and, due to device-related economic burden and risks, a refining in arrhythmic stratification strategies was needed[9]. In a recent study, *Cadrin-Tourigny et al* presented a novel arrhythmic risk prediction model (available at https://arvcrisk.com) and that was proved effective in an external experience[9,25]. To date, no specific correction for physical activity has been proposed.

This study sought to present follow up data from a highly characterized athletes ARVC cohort undergoing clinical detraining after disease diagnosis and to assess the algorithm transferability to an

external cohort of ARVC high-end athletes. The enrolled cohort of athletes practiced physical activity almost up until disease diagnosis. All practiced sport presented phases of high-end aerobic activity, when not completely endurance sports. Patient population characteristics and clinical results resulted comparable to previously presented cohorts[15,26,27], with the exception of mean age; this was due to the presence of a small number (3) of master athletes, that positively skewed our data. The patients in this cohort did not report major variation in the training regime and the overall training time over the years previous to referral and disease diagnosis, allowing the sportive history of these patient to be well characterizable.

Primary Arrhythmic Risk Evaluation in Athletes

In this cohort, the *Cadrin-Tourigny et al.* algorithm proved reliable in predicting arrhythmic events, at all analyzed follow up points. All patients used for the transferability analysis complied perfectly with the applicability criteria of the algorithm and were diagnosed with ARVC classical forms. No gross discrepancies between the *a-priori* and the observed arrhythmic risk were identified in our evaluation. The increased arrhythmic risk to which athletes are exposed seems to be already accounted for by the currently codified clinical parameters: exercise training impact in athletes may in fact already be present in a lower RVEF and higher PVC counts over the 24h, leading to a higher algorithm-predicted arrhythmic risk. Of note, our cohort underwent mandatory de-training and, therefore, a potential objection to these results could be that, were sport-induced RV remodeling in ARVC patients fully reversible, at long term follow up (e.g. at 5-years) this cohort may be more similar from an arrhythmic point of view to a non-athlete ARVC cohort than to a actively athlete (and not yet diagnosed) ARVC cohort. On this matter, some considerations should be presented.

Currently, no proof of complete reversibility of the sport-induced remodeling in ARVC patients have been presented. High-end endurance training is associated with worse long-term clinical outcomes, but

the mechanisms behind this association are not completely understood nor the long term impact of clinical detraining have been assessed[15]. Long term assessments of ARVC patients that have been practicing endurance sports and that underwent mandatory de-training are somehow limited both by sample size and length of follow up, so we believe this assessment in a specific ARVC subpopulation to be of importance for primary risk stratification purposes and for a better understanding of the disease. However, even if the sport-induced remodeling were to be completely reversible, it would still require some time after the implementation of the detraining protocol. In our cohort the perfect pattern matching between the *a-priori* algorithm predicted and the observed arrhythmic risk was assessed across the entire length of follow up, even in those early years in which a theoretically regressing but not yet-regressed remodeling should be present as an additional factor unaccounted by the algorithm. Larger case series and multicentered studies will be needed to further assess the impact and role of both endurance training and clinical detraining in ARVC patients, as well as to completely validate the algorithm in a sportsmen setting, but data from this first report seems to favor the algorithm implementation even in this sub-set of patients.

Impact of clinical detraining on patient characteristics

Clinical de-training in ARVC athlete patients was associated with a clear reduction in the 24h-PVC burden over time. A quantifiable PVC reduction trend has been presented in Figure 3. Patients presenting with a higher PVC count were generally also started on beta-blocking therapy, but as Figure 4 showed, both de-training and de-training + beta-blocker management strategy lead to a similar percentage reduction in PVC burden. Of note, PVC burden decrease appears maximum within the first six months post-detraining interval and seems to plateau at around 18 months of complete detraining, remaining stable for the rest of the follow up. However, no correlation between the reduction in PVC burden and long-term outcomes have been performed due to patient numerosity and trial structure. These authors agree with *Wang W. et al* in affirming that clinical de-training is not a strategy aimed to alter ICD-

placement decision making but should represent a therapeutic add-on[15], as also suggested by current international guidelines. Upon clinical detraining, more than half of the patients that repeated an ECG-stress test reported improvement in the arrhythmic findings during follow up as reported in Figure 5, potentially indicating a role of exercise in progressively self-eliciting exercise associated arrhythmias. While having a clear effect of PVC burden reduction and improving arrhythmic stress response, clinical de-training did not improve RVEF. At one year follow up, in most patients RVEF remained stable, with both clinically and statistically non-significant changes. Removing physical activity seemed to lead to a stabilization of the disease progression, but not to its reversion. This results do not appear to contrast with data reported by a recent study from *Chivulescu et al*[28], where a progressive deterioration of both morphological and functional characteristics at CMR of ARVC patients was observed over a long term (median of 7.0 years) follow up. The removal of physical exercise, that represented the main disease progression factor in our cohort, probably slowed, without stopping, disease progression. Additional worsening would in fact probably have been detected, if a later morphological assessment (e.g. at 5 years) had been performed. To better assess the impact of clinical detraining on RV contractility, further larger sample size assessments with longer follow up periods will be required. Of note, studying de-training effect on ventricular reverse remodeling in ARVC sub-populations with a genotype more prone to heart failure (DSG-2 for example) would be of great interest for advancing the entire field.

Outliers not following clinical de-training

Two patients were classified as outliers due to their refusal to comply to clinical de-training. In one case, the patient reached clinical criteria for ICD implantation in primary prevention, but refused to undergo the procedure. The patient kept training regularly, presenting at the following follow up visit with an increased PVC burden, several run of NSVT at Holter-ECG analysis, a drop in RVEF, and the report of several syncopal events during physical activity. ICD implantation was again proposed and patient accepted; clinical de-training was recommended and this time patient complied. PVC burden reduction

was reported twelve months later, with a RVEF value that remained stable. The second patient, implanted with an S-ICD for primary prevention, refused to comply to clinical de-training for 20 months, continuing practicing high-end endurance sports (half marathon and cycling). After assessing a significant deterioration of all clinical parameters, with a multi-disciplinary consult including family members as well, the importance of clinical de-training was understood. Its clinical scenario remained from there on stable.

Limitations

This study represents a first analysis of the novel ARVC calculator for the risk of primary ventricular arrhythmias performance in a cohort of ARVC patients with an extensive athletic background. Study limitations are mostly due to its retrospective structure and the patient follow-up protocol, although fairly standardized, was not prespecified, possibly introducing some variability in the presented results. The single center nature of the study may also contribute to generate a referral bias, being the whole cohort extracted by a third level referral center facility. Additionally, no stratification between gene-positive and gene-elusive patients was performed, due to the paucity of the sample size. Further studies with prospective evaluation and larger sample size are needed to draw certain conclusions.

CONCLUSIONS

This first analysis in an external cohort appears to validate the performance of the *Cadrin-Tourigny et al* algorithm in athletes. The practice of high-end endurance sport seem to be accounted for by the algorithm, that does not require specific adjustments. Clinical mandatory de-training has a positive effect on the PVCs 24/h burden and on dysrhythmia elicitations at ECG-stress test at mid-term follow up, while no significant reverse remodeling in RVEF was observed. Further multi-centered studies with larger sample size will be required to strengthen the obtained results.

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SUPPLEMENTARY TABLES

S-Table 1

Algorithm Eligible Patients Characteristics (n=20)	
Demographics Characteristics	
Age at Diagnosis, years (mean±s.d.)	36±14.7
Male, n (%)	17 (85.0)
Recent Cardiac Syncope, n (%)	1 (5.0)
History of NSVT, n (%)	6 (30.0)
Baseline and Holter ECG	
N of inverted T waves, n (mean±s.d)	2.3±1.6
24hPVC burden, n [R.I.Q]	1300 [450 – 3551]
RVEF, % (mean±s.d.)	47.2±8.0

S-Table2

	Predicted Sustained VA Risk per CT et al algorithm (%)	Observed Sustained VA Recurrence Rate (%)	p
At 1-year follow up	12.2 [7.5 – 16.3]	10.0	0.34
At 2-year follow up	18.5 [11.7 – 25.3]	15.0	0.29
At 3-year follow up	20.9 [13.3 – 28.4]	22.2	0.71
At 4-year follow up	24.3 [15.8 – 32.8]	25.0	0.86
At 5-year follow up	29.2 [18.5 – 39.9]	28.6	0.90

CHAPTER 5

ARRHYTHMIC RISK PREDICTION IN ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC): EXTERNAL VALIDATION OF THE ARVC RISK CALCULATOR

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Eur Heart J. *2022 Aug;2143(32):3041-3052. doi: 10.1093/eurheartj/ehac289* .PMID: 35766180

ABSTRACT

Aims: Arrhythmogenic right ventricular cardiomyopathy (ARVC) causes ventricular arrhythmias (VAs) and sudden cardiac death (SCD). In 2019, a risk prediction model that estimates the 5-year risk of incident VAs in ARVC was developed (ARVCrisk.com). This study aimed to externally validate this prediction model in a large international multicentre cohort and to compare its performance with the risk factor approach recommended for ICD use by published guidelines and expert consensus

Methods and Results: In a retrospective cohort of 429 individuals from 29 centres in North America and Europe, 103 (24%) experienced sustained VA during a median follow-up of 5.02 [2.05, 7.90] years following diagnosis of ARVC. External validation yielded good discrimination [C-index of 0.70 (95%CI, 0.65-0.75)] and calibration slope of 1.01 (95%CI, 0.99-1.03). Compared with the 3 published consensus-based decision algorithms for ICD use in ARVC (Heart Rhythm Society consensus on arrhythmogenic cardiomyopathy, International Task Force consensus statement on the treatment of ARVC and American Heart Association guidelines for VA and SCD), the risk calculator performed better with a superior net clinical benefit below risk threshold of 35%.

Conclusion: Using a large independent cohort of patients, this study shows that the ARVC risk model provides good prognostic information and outperforms other published decision algorithms for ICD use. These findings support use of the model to facilitate shared decision-making regarding ICD implantation in primary prevention of SCD in ARVC.

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a significant cause of sustained ventricular arrhythmia (VA) and sudden cardiac death (SCD), especially in young individuals and athletes. Preventing this catastrophic outcome through the prophylactic use of implantable cardioverter-defibrillators (ICDs) is a cornerstone of the disease management. Given the significant drawbacks associated with ICDs in this young and active population, appropriate patient selection is essential. Over the past 25 years, numerous studies have identified predictors of sustained VA and SCD in ARVC and consensus documents have integrated these in decision algorithms for ICD use(1-3). Building on this knowledge, a risk prediction model for sustained VA and SCD in ARVC was recently developed in a multinational cohort (n=528, designed as the derivation cohort) mostly including high volume referral centres for ARVC. (4) This prediction model provides individualized prediction of the risk of VA in patients with ARVC without a prior history of sustained VA. Since its online publication, the risk calculator's official site (www.ARVCrisk.com) has been used approximately 20,000 times illustrating its uptake in clinical practice.

The model has been internally and externally validated in small studies (4-9). However, adequately powered external validation is still lacking, (10) yet is paramount to confirm the reproducibility, generalizability, and need to update the model in an independent population.

The aims of the present study are thus 1) to conduct external validation of the published risk calculator in a distinct, adequately powered, and geographically diverse cohort including patients from six countries across North America and Europe, and 2) to compare the performance of the risk prediction model with other published guidelines and expert consensus recommendations for ICD use. During the current validation study our group detected an inaccuracy in the formula of the original ARVC risk calculator published in 2019. It was corrected both on the website (ARVCrisk.com) and in the published manuscript (REF). We base the present study on the corrected risk calculator.

METHODS

Study design

We conducted an observational, retrospective, longitudinal cohort study in accordance with the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement. (11)

Study population

The study population was derived from 29 centres (*Supplementary TableS1*) in six European and North American countries. This current cohort will be designated as the "validation cohort" while the cohort leading to the published model will be designated as the "derivation cohort". New patients from two centres participating in the original study (Montreal Heart Institute and Johns Hopkins Hospital) were included (52 patients; 12% of the cohort). No patients in the current cohort were included in the original ARVC derivation cohort. From each site, consistent with the derivation cohort, consecutive patients who (i) were diagnosed with definite ARVC as per 2010 Task Force Criteria (TFC)(12), (ii) were alive at presentation, and (iii) had not experienced spontaneous sustained VA or sudden cardiac arrest (SCA) at diagnosis were included. The study conforms to the Helsinki declaration and was approved by local ethics and/or institutional review boards. To maintain patient confidentiality, data and study materials will not be made available to other researchers for purposes of replicating the results. A limited dataset may be made available upon request.

Data collection

Data were collected independently by each of the participating centres using uniform definitions. A complete list of variables and their definitions can be found in *Supplementary TableS2*. Genetic variants were reviewed according to the American College of Medical Genetics and Genomics guidelines by cardiologists specialized in cardiovascular genetics (R.T. and J.C.T). (13)

Missing data

Patients with >50% of predictors missing were excluded from the analysis. Missingness was assumed to be at random and imputed using multiple imputation by chained equations. (14) Missing quantitative values for RVEF and LVEF were imputed manually when only qualitative assessment was available as done previously (4) and detailed in *Supplementary TableS2*. The multiple imputation model included all pre-specified predictors, proband status and genotype together with the outcome, and cumulative baseline hazard estimation. (15) A total of 25 imputed datasets were generated, and the final inference estimations were combined using Rubin's rules. (16)

Study outcomes

In accordance to the published ARVC risk prediction model which this study aims to validate, the primary outcome was the first sustained VA following the definite diagnosis as per the TFC. Sustained VA was defined as a composite of the occurrence of SCD, SCA, spontaneous sustained ventricular tachycardia (VT; lasting ≥30 seconds at ≥100 beats per minute (b.p.m.) or with haemodynamic compromise requiring cardioversion), ventricular fibrillation/flutter (VF), or appropriate ICD intervention. Heart transplantation, cardiovascular mortality, and all-cause mortality were also collected.

Predictor variables and risk calculator

The same candidate predictors as those selected in the published model based on prior literature were considered (17-19). These include sex, age, recent cardiac syncope (here defined as transient loss of consciousness and postural tone with spontaneous recovery with a likely arrhythmic mechanism, within a year of diagnosis), non-sustained VT (NSVT: defined as hemodynamically stable VT at ≥100 b.p.m., for ≥3 beats <30 s), number of premature ventricular complexes (PVCs) on 24-h Holter monitoring, extent of T-wave inversion (TWI) on anterior and inferior leads, and right ventricular ejection fraction (RVEF). Each predictor variable was determined at the time of diagnosis, defined as 1 year before to 1

year after the date of diagnosis per 2010 TFC and prior to occurrence of the primary outcome.

The 5-year risk of sustained VA for an individual patient per the published model is calculated using the following equation (4): $\mathbf{P(VA\ at\ 5\ years\ )= 1-0.8396^{\exp(LP)}}$, where the linear predictor (LP) was calculated according to the equation: LP = 0.488*male sex − 0.022*age + 0.657*history of recent cardiac syncope + 0.811*history of NSVT + 0.170*ln(24-h PVC count) + 0.113*Sum of anterior and inferior leads with TWI − 0.025*RVEF. Of note, the baseline hazard for 5 years prediction (0.8396) has been corrected since the initial publication in 2019.

Statistical analysis

Analyses were performed with RStudio version 1.3.1093 (Boston, MA, USA). Continuous variables were expressed as mean ± standard deviation or median [interquartile range (IQR)] and compared using either the independent sample *t*-test or the Mann–Whitney *U* test. Categorical variables were presented as frequencies (%) and compared using the Fisher's exact test. Follow-up duration was calculated as the time interval between the time of definite diagnosis according to TFC and the endpoint or censoring. Censoring was defined as death from any other cause, heart transplantation or the most recent follow-up visit at which the endpoint could be ascertained. Event-free survival probability was estimated using the Kaplan–Meier method and Cox Proportional Hazard regression analysis.

Model validation

The approach to external validation follows the method suggested by Royston et al. for Cox prognostic models. (20) First, the overall discriminative performance of the model was measured using Harrell's C-statistic, and the model fit by calculating the calibration slope, the regression of the LP (i.e. the product of the variable part of the Cox model) in the current cohort (validation cohort). Graphical evaluation of calibration was performed by plotting the predicted risk against the observed risk of sustained VA, using

grouped Kaplan–Meier estimates and the continuous hazard regression function. The choice of the number of groups presented was based on the balance between providing sufficient spread in group risk, while maintaining adequate group sizes for precision. For the complete cohort, 5 groups are presented while for subgroup analyses, 4 groups are presented.

Subsequently, a more in-depth analysis of the model fit was performed by a Cox's model including the same predictor variables in combination with the LP of the original model (as an offset variable) to evaluate potential differences in the regression coefficients of each individual predictor. The result indicating the validity of the model would be that if all coefficients β^* equalled 0, reflecting that all the variability in the validation sample is accounted for by the published model. In addition, the baseline survival function of the validation dataset was compared to that of the derivation dataset to see if the overall predictions need to be globally shifted upward or downward. Lastly, a new prediction model using the same predictor variables was fitted to the validation dataset and compared to the fit of the original model using the Akaike Information Criterion (AIC), with a difference of >2 defined as statistically significant. This allows testing whether a model specifically fitted to the validation dataset performs better than the original model in the validation dataset.

Subgroup analyses

We visually explored the performance of the model specifically in different populations of interest by comparing calibration plots for these subgroups. We stratified the cohort by geographic origin (Europe vs North America), by proband status and by Plakophilin 2 carrier status (*PKP2*; causal variant carrier vs non-carrier). We did not report quantitative markers of performance such as the C-statistic as this study was not powered adequately for these subgroups.

To assess the impact of carrying an ICD on prediction accuracy, we also presented calibration plots based on ICD carrier status at baseline defined as ICD implantation prior to a year following diagnosis and first VA outcome, whichever came first.

Clinical utility

To assess the relative clinical utility of the risk prediction model, it was compared to 3 other published expert consensus algorithms for ICD implantation in ARVC: The 2015 International Task Force Consensus for the treatment of ARVC (ITFC) (17), the 2017 American Heart Association (AHA) guidelines for the management of VA and prevention of SCD (2) and the 2019 Heart Rhythm Society (HRS) consensus on arrhythmogenic cardiomyopathy (excluding programmed ventricular stimulation) (21) through decision curve analysis. In a decision curve analysis (22), the clinical benefit is assessed by the "net benefit" representing the balance between useful (i.e in patients with events) vs useless (i.e. in patients without events) ICD placement at 5 years weighted according to the threshold used for ICD implantation. More specifically, the decision curve uses the following formula: **True positives / Total Sample Size – False positives / Total Sample Size * (pt/1-pt)**

Where "pt" represents threshold probability, in the current case, threshold for ICD implantation. Therefore, the higher the threshold used, the greater the harm of useless ICD use (i.e. false positive) is valued. Higher values indicate greater benefit while a value of 0 indicates no benefit.

To present the consequence of setting different thresholds for ICD implantation, we evaluated and plotted the proportion of patients who would receive ICDs and the proportion of treated and missed events at each threshold. We compared these with the recommendations for ICD use by the 3 published consensus mentioned above (ITFC(1), AHA(2), HRS(3)).

RESULTS

The study population included 429 definite ARVC patients without a history of sustained VA or SCA at the time of diagnosis aged 43.1± 15.8 years and slightly more than half ($n = 235$, 54.8%) were male. Probands accounted for two-thirds of the cohort ($n = 278$, 64.8%). Half ($n = 198$, 46.6%) of patients had a pathogenic or likely pathogenic variant in a gene with definite or moderate association with ARVC

(23), which represents 70% (198 patients) of the 282 patients for whom the complete genetic information was available. *PKP2* was the most common genotype, carried by 111 patients (26%) followed by DSP in 38 patients (9%). Compared to PKP2 patients, DSP patients were more likely to have a decrease in LVEF<50% (44.7% vs 6.4%) but less likely to have VA events at follow-up (13.2% vs 24.5%). Baseline characteristics according to genotype are presented in *in Supplementary TableS3.* Other clinical and demographic characteristics are summarized in *Table1. Baseline characteristics by country of origin are presented in TableS4, and a* comparison of the derivation and validation cohort populations is presented in *TableS5*.

Table 1. Baseline clinical characteristics

	Overall (n=429)	No sustained VA (n=326)	Sustained VA (n=103)	P value
Demographics and genetics				
Age at diagnosis (years)	43.1 ±15.8	44.1±15.7	40.1±16.0	0.025
Male sex	235 (54.8)	159 (48.8)	76 (73.8)	<0.001
Proband status	278 (64.8)	197 (60.4)	81 (78.6)	0.001
(Likely) pathogenic variants (n= 282)	198 (46.2)	150 (46.0)	48 (46.6)	0.480
Genotype				0.302
PKP2	111 (25.6)	84 (25.8)	27 (26.2)	
DSP	38 (8.9)	33 (10.1)	5 (4.9)	
DSG2	27 (6.3)	22 (6.7)	5 (4.9)	
DSC2	3 (0.7)	1 (0.3)	2 (1.9)	
JUP	0 (0.0)	0 (0.0)	0 (0.0)	
TMEM43	10 (2.3)	4 (1.2)	6 (5.8)	
PLN	3 (0.7)	3 (0.9)	0 (0.0)	
Multiple mutations	6 (1.4)	3 (0.9)	3 (2.9)	
Clinical history				
Recent cardiac syncope (n=424)	37 (8.6)	16 (4.9)	21 (20.4)	<0.001
ECG/continuous ECG monitoring				
TWI in ≥ 3 precordial leads (n=409)	250 (58.3)	187 (57.4)	63 (61.2)	0.295
TWI in ≥ 2 inferior leads (n=403)	109 (25.4)	81 (24.8)	28 (27.2)	0.589

PVC count (n=324)	1434 [439-3601]	1354 [400-3719]	1676 [602-3492]	0.160
NSVT (n=359)	148 (34.5)	105 (32.2)	43 (41.7)	0.001
Imaging				
RVEF (%) (n=410)	45 [36-53]	47 [38-53]	40 [35-48.5]	<0.001
LVEF (%) (n=404)	57 [51-60]	57 [51-61]	57 [50-60]	0.049
Treatment at baseline				
ICD	175 (40.8)	113 (34.7)	62 (60.2)	<0.001
β-blockers (n=407)	206 (48.0)	156 (47.9)	50 (48.5)	0.50
Follow-up	5.02 [2.05-7.90]	4.48 [1.86-7.32]	6.12 [2.60-10.08]	0.002

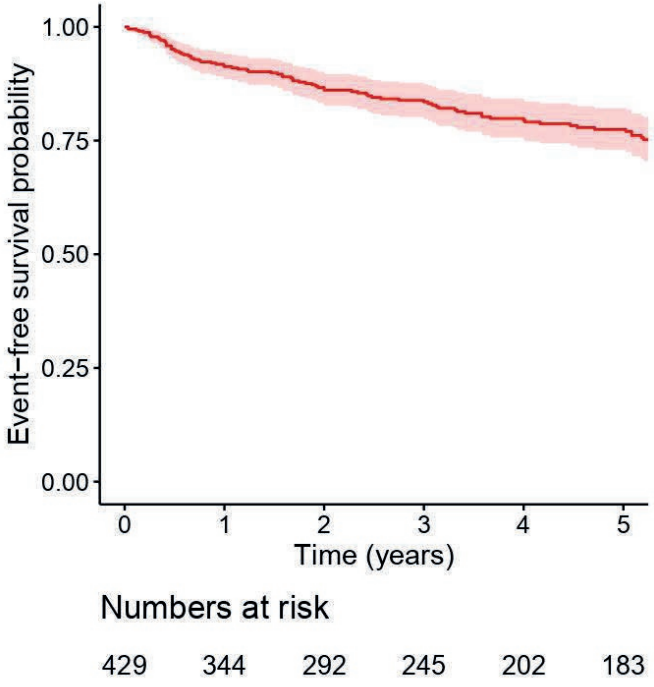
Variables are expressed as frequency (%), mean±standard deviation, or median [IQR]. Total number of pts with available data for a variable are mentioned in parenthesis. DSG2, desmoglein-2; DSP, desmoplakin; ICD, implantable cardioverter-defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; NSVT, non-sustained ventricular tachycardia; JUP, junction plakoglobin; PKP2, plakophilin-2; PLN, phospholamban; PVC, premature ventricular complex; RVEF, right ventricular ejection fraction; TMEM43, Transmembrane Protein 43; TWI, T-wave inversion; VA, ventricular arrhythmia.

Overall, 299 (70.0%) patients had complete data for the pre-specified predictors. Six of the eight predictors had missing data: recent cardiac syncope ($n = 5$, 1.17%), NSVT (70 = 16.32%), PVC count ($n = 105$, 24.48%), extent of leads with TWI ($n = 26$, 6.06%) and RVEF ($n = 19$, 4.43%). From an initial cohort of 433 patients, four patients were excluded as >50% of their predictors were missing (4 predictors or more).

Outcomes

During a median follow-up of 5.02 [2.05-7.90)] years, 103 patients (24%) experienced sustained VA events corresponding to an annual event rate of 4.98% (95% confidence interval (CI): 4.07-6.04). **Figure1** shows the cumulative survival free from first sustained VA.

Figure 1



Survival-free from sustained ventricular arrhythmia at follow-up. The cumulative event-free survival for any ventricular arrhythmia with 95% confidence intervals (shaded area) is plotted.

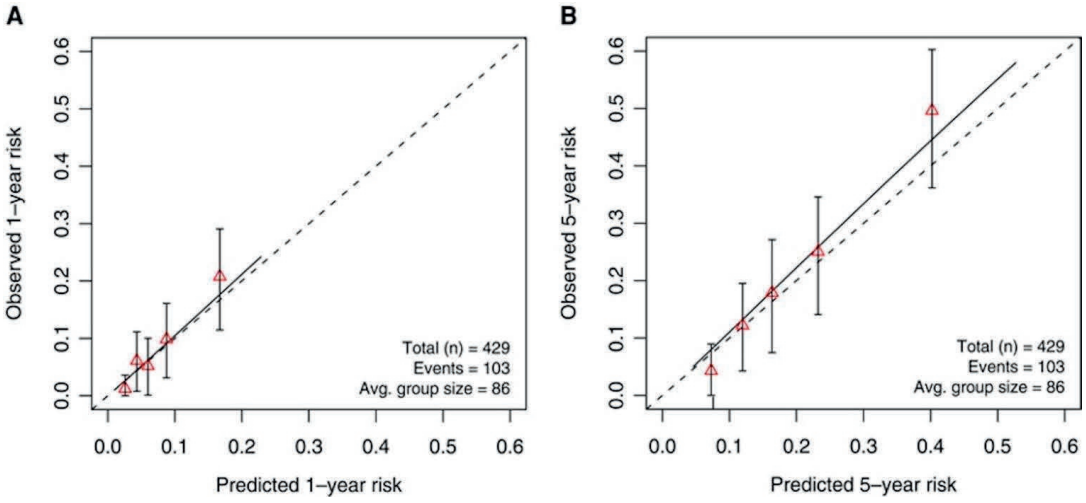
Among patients who experienced sustained VA during follow-up, the most common events were ICD treated VAs, which represented 59.2% of events (*n* =61), followed by sustained VT (*n* = 32, 31.1%), SCA (*n* =7, 6.8%) and SCD (*n* =3, 2.9%). In patients with sustained or ICD treated VT events, the median cycle length (available in 57/93 events) was 280 ms (IQR: 246-315) which corresponds to 214 bpm (190-243). At last follow-up, 9 (2.1%) patients had died, including 2 from non-cardiac causes, and 7 (1.6%) had undergone heart transplantation.

External validation

Model validation revealed a Harrell C-index of 0.70 (95% CI 0.65-0.75). The calibration slope was

1.01 (95% CI 0.99-1.03) showing no significant difference in discrimination. The calibration of the model is graphically presented in **Figure 2** demonstrating good overall agreement between predicted and observed shorter-term (1-year) and longer-term durations (5-year) with no significant over or under prediction across the complete risk spectrum. The distribution of patients according to their risk is presented in supplementary figureS1 and calibration plots for intermediate durations (1,2,3 and 5 years) in supplementary FigureS2.

Figure 2



Calibration plots presenting the agreement between predicted (x axis) and observed (y axis) 1-year and 5-year risk of ventricular arrhythmia (VA). Triangles represent binned Kaplan–Meier estimates with 95% confidence intervals for quintiles of predicted risk. The straight line is the continuous calibration hazard regression with the dotted line represents optimal calibration (i.e. perfect correspondence between predictions and observations across the risk spectrum). The calibration is shown to be acceptable across the risk spectrum with no significant under or over prediction in any risk category. VA, ventricular arrhythmia.

Two different aspects of the model fit or potential misspecification were evaluated. First, the assessment of individual predictor coefficients (**Figure 3**, **panel A)** all showed no significant diversion from the original model in this cohort. This finding means that none of the individual coefficient would benefit

from being modified from their original values to improve prediction in this cohort. Second, the baseline survival function (i.e. predictors-adjusted survival) was assessed through the comparison of the baseline survival probabilities (i.e. predictors-adjusted survival) in the derivation and the validation cohorts at different time points showing similar expected survival curves as shown numerically and visually in **Figure 3, Panel B *and C***. These findings suggest that the survival function does not need to be modified to improve prediction in this cohort. Finally, the potential need to update the model was assessed by comparing the fit of the published model with the derivation of a new model in the validation cohort. The AIC of the published model in the current cohort (1059.14) and of a model derived in this cohort (1060.93) were not significantly different (absolute difference in AIC of 1.79) indicating the absence of significant improvement in predictions when fitting a model to this population. As a sensitivity analysis, we repeated the process in patients with complete data (N=299) resulting in a similar C-statistic, calibration slope, baseline risk and calibration plot (Supplementary FigureS3).

Clinical utility

We compared the performance of the risk calculator with published consensus-based decision algorithms for ICD use in ARVC. As illustrated in *Figure 4*, the risk calculator generally had a superior net clinical benefit when compared to the other published algorithms for ICD use. Its performance becomes similar to the HRS consensus above a risk of approximately 35%. Finally, we graphically presented the impact of different threshold for ICD implantation on the proportion of ICD use and the protection rate and compared to the published decision algorithms (Figure 5). Higher thresholds result in less ICD use but less protection from VA. As an example, a threshold of 15% would results in implanting 59.4% of patients with ICDs while protecting 85.7% of patients with incident VA events.

Figure 3

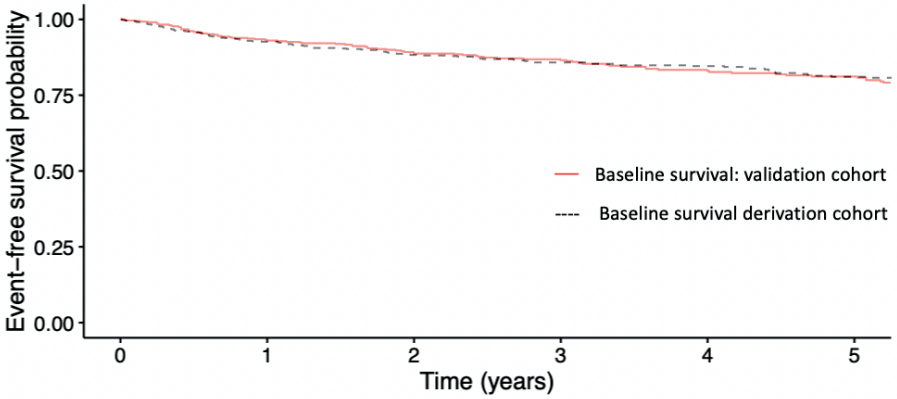
A

	Individual predictor coefficients	
Predictors	Coefficient	P value
Sex	0.305	0.188
Age	0.013	0.051
PVC count	-0.117	0.595
NSVT	-0.361	0.171
Leads with TWI	-0.062	0.156
Cardiac Syncope	0.321	0.225
RVEF	0.001	0.946

B

	Probability of survival	
Time	Derivation cohort	Validation cohort
1 year	0.938	0.938
2 years	0.901	0.899
3 years	0.880	0.880
4 years	0.870	0.850
5 years	0.840	0.831

C



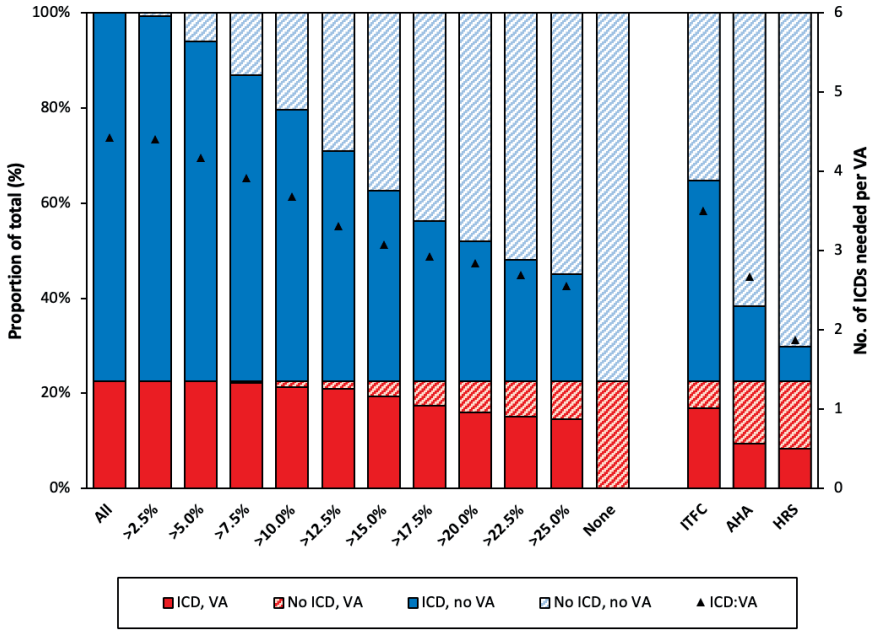
Assessment of the model fit. Assessment of the individual predictors (Panel A) show an absence of diversion from the initial model as all coefficients are non-significantly different from 0. Compared survival probability of the derivation and validation cohorts (Panel B) and baseline survival hazard (i.e. predictors-adjusted survival) presented as survival curves (Panel C) both show similar expected survival. NSVT, non-sustained ventricular tachycardia; PVC, premature ventricular complex; TWI, T-wave inversion; RVEF, right ventricular ejection fraction.

Subgroups analyses

The performance in subgroups of interest was visually explored by calibration plots presented in *Supplementary figureS4*. This cohort was not sufficiently powered to provide definite answers in these subgroups. Calibration appeared acceptable in patients from both Europe and North America, although this analysis had low precision in the North American population due to its smaller size. The model performed well both in probands and family members with a possible trend toward overestimation in family members in the lower risk spectrum. The calibration was also visually acceptable both in *PKP2* carriers and non-carriers. Calibration plots according to the presence of an ICD show an acceptable

agreement between predictions and observations with a tendency towards overestimation in non-ICD carriers and underestimation in ICD-carriers in the higher risk spectrum (*Supplementary figure S5*).

Figure 5



	VA	ICD	>2.5%	>5.0%	>7.5%	>10.0%	>12.5%	>15.0%	>17.5%	>20.0%	>25.0%	ITFC	AHA	HRS
True negative	-	-	0.7%	6.0%	13.1%	20.4%	29.0%	37.3%	43.8%	48.0%	54.9%	35.3%	61.7%	70.1%
False positive	-	+	76.7%	71.4%	64.4%	57.0%	48.4%	40.1%	33.6%	29.4%	22.5%	42.1%	15.8%	7.3%
False negative	+	-	0.0%	0.1%	0.5%	1.3%	1.6%	3.2%	5.1%	6.6%	8.1%	5.7%	13.2%	14.3%
True positive	+	+	22.6%	22.5%	22.1%	21.3%	21.0%	19.4%	17.4%	16.0%	14.5%	16.9%	9.4%	8.3%
ICD:VA			4.4	4.2	3.9	3.7	3.3	3.1	2.9	2.8	2.6	3.5	2.7	1.9
Sensitivity			100.0%	99.7%	98.0%	94.3%	93.1%	85.7%	77.2%	71.0%	64.3%	74.8%	41.7%	36.9%
Specificity			0.9%	7.7%	16.9%	26.3%	37.4%	48.2%	56.6%	62.0%	70.9%	45.6%	79.6%	90.6%
ICD proportion			99.3%	93.9%	86.5%	78.3%	69.5%	59.4%	51.0%	45.5%	37.1%	59.0%	25.2%	15.6%
Protection rate			100.0%	99.7%	98.0%	94.3%	93.1%	85.7%	77.2%	71.0%	64.3%	74.8%	41.7%	36.9%

Impact of implantable cardioverter defibrillator use threshold on clinical outcomes. The potential impact of different thresholds for ICD use according to the model is presented on the left side and the proportion of patients who would get an ICD according to the different consensus statements is presented on the right side. For each threshold (x axis) the proportion of patients (y axis) who have events (red) who don't have events (blue), who would receive an ICD (solid colors) or not receive one (hashed colors) are presented. The triangles represent the number of ICD needed per event prevented for each threshold (right sided y axis). The numerical values are presented in the table below. ICD:VA, ratio of implantable cardioverter-defibrillator placements required to protect one patient developing ventricular arrhythmia; other abbreviations as per Figure 4.

DISCUSSION

In this study, we validated the published ARVC risk calculator in an independent cohort of patients from 29 centres in 6 countries in North America and Europe. Since its publication in 2019, the risk calculator had a significant uptake in clinical practice. Ensuring its reproducibility and accuracy in an independent patient population is crucial to ensure both usefulness and safety. The main findings are as follows:

1) Demonstration that the model is accurate in its predictions with an adequate discrimination and calibration in a cohort with a sufficient sample size. (10, 24) The performance of the risk calculator was indeed comparable to what was reported initially and its prediction accuracy in this cohort would not be improved by recalibration. (20)

2) Demonstration that the risk calculator generally outperforms various risk factor approaches recommended in published consensus-based algorithms for ICD use in ARVC.

These findings thus support the clinical use of this risk prediction model as a valuable tool for sustained VA and SCD risk stratification in definite ARVC and, consequently, for guiding decisions about primary prevention ICD indications.

Comparison of the Internal and External Validation Populations

While based on the same inclusion criteria (i.e. definite diagnosis of ARVC and no prior history of sustained VA at the time of diagnosis), the initial risk calculator included a high proportion of patients treated at highly specialized ARVC referral centres. Thus, a significant concern regarding this population is a possible selection bias due to the preferential referral of patients for adverse disease progression (i.e recurrent ventricular arrhythmia referred for ablation and severe heart failure for advanced therapies). This could potentially hamper external validity. The present cohort derived from 29 different centres in 6 countries is thus likely to reflect a more diverse ARVC population. Expectedly, the annual event rate

in this validation cohort (4.98%, 95%CI 4.07-6.04) was slightly lower, although non-significantly, than in the derivation population (5.6%, 95%CI 4.7–6.6) during a similar follow-up period (5.02 [2.05-7.90] years in the validation versus 4.83 [2.44-9.33] years in the derivation cohort]. This reflects the overall high risk of VA events in definite ARVC patients such as those included in this study which is consistent with prior literature and often preceding structural changes.(4, 18, 25-28)

Some differences between the two cohorts (shown in *TableS4*) might have limited the potential discrepancy in event rates, such as a higher proportion of probands (64.8% vs 49.8%, p<0.001) and males (54.8% vs 44.7%, p=0.002) in the current cohort. Conversely, patients in the present cohort were slightly older (43.1 vs 38.2 years of age, p<0.001), had less recent cardiac syncope and NSVT. The proportion of patients with decreased LVEF (<50%) was also higher in this cohort (17.7 vs 12.7, p=0.002). Although individuals in the current population were more likely to receive anti-arrhythmic drugs (p<0.001) and β-blockers (48.0 vs 37.9, p=0.001), the proportion of ICD carriers at baseline was similar (41.1 vs 40.8 p=0.98). Finally, while still representing the predominant genotype, the proportion of patients with *PKP2* causal variants was lower than in the derivation cohort (39.4% vs 51.1% of tested patients) factoring that the current cohort has a lower proportion of patients with known genetic information. This predominance of PKP2 genotype is consistent with prior literature including patients with definite ARVC diagnosis.(29) The proportion of patients with DSP causal variants was also higher (8.9% vs 4.4%) than in the derivation cohort.

Model Performance

The current validation cohort included 429 patients, of whom 103 had events. This met the minimally recommended sample size of 100 patients with and 100 patients without events to attain sufficient power for external validation. (24) The initial study and internal validation using bootstrapping yielded an optimism corrected C-statistic of 0.77 (95% CI 0.73-0.81) and a calibration slope of 0.93 (95% CI 0.92-0.95). In the current study, we obtained comparable results with a slightly lower C-statistic of 0.70 (95%

CI 0.65-0.75) showing acceptable discrimination and a calibration slope of 1.01 (95% CI 0.99-1.03) demonstrating almost perfect agreement between predictions and observations for sustained VA. As illustrated in the calibration plot, this concordance between observations and predictions was consistent across the risk spectrum (*Figure2*). Calibration in subgroups based on geographical origin, pedigree position, and genotype did not reveal major discrepancies although the study was not adequately powered to arrive at definitive conclusions in these subgroups.

The results of the current study are consistent with five small studies which have addressed the external validation of the ARVC risk calculator since its publication. The risk calculator was shown to perform well in patients with a definite diagnosis of ARVC (5, 6, 8) and regardless of their exercise status (7). The validation study by Baudinaud *et al*, on a cohort of 115 patients, only 15 with VA events, of whom only one had an ICD at baseline, reported a C-statistic of 0.84 (CI 0.74-0.93) while reporting an overestimation of the risk in lower risk patients. (6)

Clinical utility

The model generally showed a superior net clinical benefit when compared to a risk factor approach as recommended in the three published consensus documents(2, 17, 21). The model was similarly shown to outperform the ITFC and HRS consensus in two separate cohorts (5, 6). These studies, however, suggested highly different thresholds for ICD implantation (10% and 37%), assuming an equal weight to unprotected VA and unnecessary ICDs. We did not present such an analysis as we do not propose that these adverse events are equivalent and rather preferred the use of the weighted analysis along with the graphical presentation of the clinical implications of different threshold. The question of the threshold for ICD implantation is a legitimate concern when using the risk calculator. Establishing a single perfect threshold is a delicate undertaking as every cut-off point comes with a trade-off between unnecessary ICDs with their potential complications versus the potential for unprotected SCA. The relative weight of

these opposing undesirable events varies significantly from one individual to another. In the individualized decision-making process however, a few points should be considered when reflecting on the threshold for ICD use. First, when tempted to use a similar threshold as suggested by the guidelines for the hypertrophic cardiomyopathy (HCM) risk calculator (i.e. ≥6% within 5 years) (30, 31), the breakdown of the type of events is relevant. In ARVC cohorts, including the current study and in the derivation cohort, most events were either ICD treated events or sustained VA, while most events in the cohort leading to the HCM risk calculator cohort were SCD or SCA.(32) Although most clinicians agree that sustained or ICD treated VAs represent significant events, supported by guidelines(2, 33), the exact number of treated VA events corresponding to a potential SCD is unknown in ARVC. Another important aspect to consider is that none of these studies are prospective evaluations of the role of ICDs in SCD prevention. Such an undertaking would not be feasible in contemporary high-risk ARVC populations. However, from such prior studies in the general cardiomyopathy population the one which established a benefit for primary prevention ICDs with the lowest annual risk of mortality, SCD-HeFT, had an annual risk of SCD of 3.5%/year. (34) Finally, the cost of ICDs is rarely a significant determinant nowadays in countries where ICDs can be considered in primary prevention.(35) Factoring the low number of ICDs needed to treat one VA event in ARVC, decreases in cost of devices, the lifespan of modern ICDs reaching 10 years, and the potential number of quality adjusted live years (QALY) saved in this young, usually otherwise healthy population (only 5 individuals had non arrhythmic death during follow-up in this cohort), the common, although debated thresholds for a QALY between 50,000-100,000 USD (36) remains far of reach. Conversely, the rate of short- and long-term complications of ICDs remain significant in ARVC patients (annual rate of complications of 4.2% and of inappropriate shocks of 3.9%) (37), and although subcutaneous-ICDs have become an appealing alternative, there is no evidence of a lesser risk. (38, 39)

Thus, in light of these different considerations, we do believe that the best use of the risk calculator is as

a shared-decision making tool balancing the opposing risks of SCD and ICD use. It appears reasonable that the predicted 5-year risk threshold for recommending an ICD would range from 5-25%, depending on the patient's values and preferences, and the clinician's judgement. We acknowledge that the threshold may change in the future with advances in non-invasive treatments and innovations in ICD technology which may lower risks associated with devices.

Future improvements in the model

While the model demonstrated a better performance compared to other published decision algorithms, it remains imperfect as illustrated by a C-statistic of 0.70. While it is unlikely that any risk stratification tool for SCD could predict the totality of these events, different elements could potentially improve prediction in the future. The addition of more refined parameters indicating LV involvement, including late gadolinium enhancement were recently suggested. (9) Genotype may also improve SCD risk prediction as recently proposed for patients with Phospholamban associated disease (40). Finally, additional invasive parameters such as programmed ventricular stimulation (41, 42) might add additional accuracy in intermediate risk cases. Moreover, the model is based on prediction of risk from the time of diagnosis of ARVC; a time-updated model for repeated risk prediction may have practical clinical utility.

LIMITATIONS

In this study, the majority of sustained VA outcomes are ICD treated events. While this fact is not possible to overcome in most modern ARVC populations and while most would agree that these still represent significant events, they do not directly represent the underlying risk of SCD. However, this is a limitation shared with most of previous studies in this field, including most of those used to elaborate prior consensus-based risk-stratification algorithms. While underpowered for events, calibration plots by ICD carrier status show acceptable correlation between predictions and observations. This reflects both

that ICDs are implanted in patients believed to be at higher risk (selection bias), but also increase the detection of some arrhythmia that might have gone undetected otherwise (information bias). (*Supplementary material online, FigureS4*). While family members are well represented in the derivation cohort (50.2%), they are less prevalent in the current cohort (35.2%) and contribute to a lower proportion of events (21.1%). The calibration plot in this specific subgroup, although underpowered, suggests possible overestimation in the lower risk patients which should be taken in consideration when using the model. Missing data also represents a limitation of this retrospective cohort. Although a complete case analysis reassuringly demonstrates similar results with regards to performance, missing data could influence the relative benefit of the model over consensus-based methods. Finally, this validation only applies to patients who were well represented in the derivation and validation cohorts. The model should thus not be used in patients who do not meet definite ARVC diagnosis per 2010 TFC such as those with left dominant forms and in patients with rare malignant genotypes such as *TMEM43*-p.S358L, of which only 10 patients were included in this cohort.

CONCLUSION

In this external validation study, we demonstrated that the published ARVC risk prediction model not only provides accurate prognostic information in patients with ARVC without a prior history of sustained VA at diagnosis, but also performs generally better than other published decision algorithms. These findings support its clinical use as a valuable tool for risk stratification enabling consistent and effective shared decision making for ICD implantation.

Funding

P.J. is supported by the Daniel Bravo Foundation grant and Spanish Society of Cardiology Magda Heras mobility grant, A.G. by the Wilton W. Webster Fellow of Heart Rhythm Society, The Johns Hopkins ARVD/C Program by the Leonie-Wild Foundation, Leyla Erkan Family Fund for ARVD Research, Hugh Calkins, Marvin H. Weiner, and Jacqueline J. Bernstein Cardiac Arrhythmia Center, Dr. Francis P. Chiramonte Private Foundation, Dr. Satish, Rupal, and Robin Shah ARVD Fund at Johns Hopkins, Bogle Foundation, Campanella family, Patrick J. Harrison Family, Peter French Memorial Foundation, and Wilmerding Endowments, H.K.J. by grants from Novo Nordisk Foundation, Denmark (NNF18OC0031258 and NNF20OC0065151), S.A.L. by NIH grant 1R01HL139731 and American Heart Association 18SFRN34250007, R.T by the Canada Research Chairs program, J.C.T by the Philippa and Marvin Carsley cardiology research chair.

Conflicts of interest

C.M: honoraria from Abbott, I.O.: grants, consulting fees or honoraria from BSM, Cytokinetics, Shire, Genzyme, Amicus, Menarini International and Boston Scientific and Tenaya, S.A.L.: grants from BMS/Pfizer, Boehringer Ingelheim, fitbit, IBM, and consulting fees from BMS/Pfizer, Invitae and Blackstone, J.S.H.: research grants from Boston Scientific, Abbott and Medtronic and is on Scientific Advisory Board for Boston Scientific, H.K.J: grant from Novo Nordisk and honoraria from Abbott and Biosense Webster, C.A.J.: consulting fees from Pfizer, J C-T. consulting fees from BMS/Pfizer and Bayer.

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Supplementary Material

Supplementary tables

Table S1: Centres and investigators that participated in the inclusion

Country	Principal investigator(s)	Included hospitals
Canada	Dr Julia Cadrin-Tourigny Dr Jason D Roberts Dr Ciorsti Maclntyre Dr Colette Seifer Dr Wael Alqarawi Dr Jeffrey S. Healey Dr Andrew D. Krahn	- Montreal Heart Institute (Montreal) - Western University Hospital (London) - Hamilton General Hospital/McMaster University (Hamilton) - British Columbia inherited arrhythmia clinic (Vancouver) - Dalhousie University and Queen Elizabeth II Health Sciences Centre (Halifax) - St-Boniface General Hospital, University of Manitoba (Winnipeg) - Ottawa Heart Institute (Ottawa)
Denmark	Dr Henrik Kjaerulf Jensen	- Aarhus University Hospital (Aarhus)
France	Dr Jean-Baptiste Gourraud Dr Antoine Delinière Dr Philippe Chevalier	- CHU Nantes Hospital database (Including CHU Nantes, CHU Bordeaux, CH de Grasse, CHU Clermont-Ferrand, CHU Limoges, Hôpitaux Universitaires de Marseille, CHU Montpellier, CHU Rennes, CHU Toulouse) - Hôpital Cardiovasculaire Louis Pradel (Lyon)
Italy	Dr Claudio Tondo Dr Andrea Mazzanti Dr Leonardo Calò Dr Silvia Giuliana Priori Dr Michela Casella Dr Iacopo Olivotto	- Centro Cardiologico Monzino (Milan) - Istituti Clinici Scientifici Maugeri IRCCS (Pavia) - Careggi University Hospital (Florence) - Policlinico Casilino (Rome) - University Hospital Salesi-Lancisi (Ancona)
Spain	Dr Zoraida Moreno Weidmann Dr Andrea Di Marco Dr Elena Arbelo	- Hospital de la Santa Creu i Sant Pau (Barcelona) - Hospital Universitari de Bellvitge (Hospitalet de Llobregat) - Hospital Clinic de Barcelona
United States of America	Dr Steven A Lubitz Dr Michael J. Cutler Dr Hugh Calkins Dr Cynthia A. James	- Massachusetts General Hospital - Intermountain Medical Center Heart Institute - The Johns Hopkins Hospital

Table S2: Variables definitions

Name of the variable	Description and Definition
Choices for coding and their definitions	
Patients Characteristics	
ARVDNu	ARVD database number
Number	
Site	Site of enrolment
YOB	Date of birth
yyyy	
Sex	Gender of patient
Male =1 Female = 0	
Pedigree	Proband or family member
1=Proband 2=Family member **Proband definition:** first affected family member seeking medical attention for ARVD/C in whom the diagnosis was confirmed (i.e. an individual ascertained independently of family history).	
Ancestry	Ancestry of the patient
1=Caucasian, 2= African, 3=Asian 4=other	
Mutation	Pathogenic mutation associated with ARVD/C detected. **Definition:** Pathogenic or likely pathogenic mutation according to ACMG guidelines (1) in one of the genes associated with ARVC (PKP2, DSP, DSG2, DSC2, JUP, TMEM43, PLN).(2) .
1=yes, 0=no	
Gene	Gene with mutation
1=*PKP2,* 2=*DSP*, 3=*DSG2*, 4=*DSC2*, 5=*JUP*, 6=*TMEM43*, 7=*PLN*, 8=CH/HO/DG (CH: compound heterozygous mutations; DG: digenic mutations; HO: homozygous mutations) 9=other (describe in genetic remarks)	
Amino acid	Amino acid change(s)
Text	
DNA change	Nucleotide changes (cDNA)
Text	
Genetic remarks	Additional genetic screening/remarks
Text	
CH/HO/DG_explanation	If gene=8: indicate if CH, HO or DG and provide details of second pathogenic variant
Text	

Variables at diagnosis

One year before or year after diagnosis and before the first event

Prioritize exams in the 1-year time frame before and after diagnosis. If not available, code the next most recent exam available

DateofDx	Date at which definite ARVC was attained according to 2010 Task force criteria (TFC): 2 major criteria (from 2 different categories) 1 major and 2 minor criteria (from 3 different categories) 4 minor criteria (from 4 different categories)
dd-month-yyyy	
CardiacSyncopeDx	**Definition:** *Transient loss of consciousness and postural tone with spontaneous recovery with* ***arrhythmic mechanism likely at diagnosis. This thus excludes syncope of vaso-vagal etiology.*** (1 year before/after Dx and before the first event)
1=yes, 0=no	
DateCardiacSyncopeDx	Date of cardiac syncope closest to time of Dx
dd-month-yyyy	
RecentCardiacSyncopeDx	Definition: Cardiac syncope defined as above which occurred within 6 months prior to diagnosis to 1 year after diagnosis and prior to event.
1=yes, 0=no	
DateRecentCardiacSyncopeDx	Date of recent cardiac syncope closest to time of Dx
dd-month-yyyy	
1=yes, 0=no	
ECGdx	ECG performed at diagnosis (1 year before/after Dx and before the first event)
1=yes, 0=no	
DateECGDx	Date of ECG: select ECG with the most leads with T-wave inversion within 1 year of diagnosis and prior to first event.
dd-month-yyyy	
BBBDx	Presence of bundle branch block (on ECG selected for "DateECG")
0=no **1**=Right Bundle branch block **(RBBB):** 1- QRS duration greater than or equal to 120 ms in adults, greater than 100 ms in children ages 4-16 years and greater than 90 ms in children less than 4 years of age. 2- rsr' rsR' or rSR' in leads V1, or V2. The R' or r' deflection is usually wider than the initial R wave. In a minority of patients, a wide and often notched R wave pattern may be seen in lead V1 and/or V2. 3- S wave of greater duration than R wave or greater than 40 ms in leads I and V6 in adults. 4- Normal R peak time in leads V5 and V6 but greater than 50 ms in lead V1. Of the above criteria, the first 3 should be present to make the diagnosis. When a pure dominant R wave with or without a notch is present in V1, criterion 4 should be satisfied. Definitions from: AHA/ACCF/HRS Recommendations for the Standardization and Interpretation of the Electrocardiogram 2009 (3)	
NumLeads_Tinversion_antDx	Number of precordial leads with T-wave inversion (V1 through V6). (on ECG selected for "DateECG") **Definition:** *T-waves are considered inverted if amplitude ≥ 1 mV (1 mm).*
Number	
NumLeads_Tinversion_infDx	Number of inferior leads with T-wave inversion II, III and AVF. (on ECG selected for "DateECG") **Definition***: T-waves are considered inverted if amplitude ≥ 1 mV (1 mm).*
Number	
1=yes, 0=no	
ECG_Comments	Comments on ECG
text	
HolterDx	Was Holter performed at diagnosis? (1 year before/after Dx and before the first event)
1=yes, 0=no	

MaxHolterPVCcountDx	Maximum PVC count on a 24 hrs Holter (1 year before/after Dx and before the first event)
Number	
DateHolterDx	Date Holter with maximal PVC count performed (1 year before/after Dx and before the first event)
dd-month-yyyy	
NSVTDx	History of non-sustained VT (NSVT) on any exam at diagnosis (Any time before diagnosis up to one year after dx and before the first event). **Definition of NSVT:** 3 or more consecutive ventricular beats at a rate of >100 beats per minute with duration of less than 30 seconds and without hemodynamic compromise.
1=yes, 0=no	
DateNSVTDx	Date of the test where NSVT was first reported (Any time before diagnosis up to one year after dx and before the first event).
dd-month-yyyy	
ECHODx	Transthoracic echocardiogram performed at diagnosis? (1 year before/after Dx and before the first event)
1=yes, 0=no	
DateECHODx	Date transthoracic echocardiogram performed: N.B. *If a patient has more than one exam with the same imaging technique, the exam with the most complete and reliable report that is the closest from the date of diagnosis will be selected for coding.* Prioritize 1-year time frame before and after dx).
dd-month-yyyy	
RV_FAC_Dx	Right ventricular (RV) fractional area change on transthoracic echocardiogram. *(on Echo chosen for DateECHODx)*
%	
MRI_Dx	Magnetic resonance imaging (MRI) performed at diagnosis? (1 year before/after Dx and before the first event)
1=yes, 0=n	
DateMRIDx	Date MRI performed (1 year before/after Dx and before the first event). *If a patient has more than one exam with the same imaging technique, the exam with the most complete and reliable report that is the closest from the date of diagnosis will be selected for coding.* Prioritize 1-year time frame before and after dx.
dd-month-yyyy	
AngioDx	RV angiogram performed at Diagnosis
1=yes, 0=no	
DateAngioDx	Date RV angiogram was performed (1 year before/after Dx and before the first event) *If a patient has more than one exam with the same imaging technique, the exam with the most complete and reliable report that is the closest from the date of diagnosiswill be selected for coding.* Prioritize 1-year time frame before and after dx.
dd-month-yyyy	
RVEFECHO_Dx	RV ejection fraction (RVEF) as measurement for RV dysfunction on transthoracic echo *(on Echo chosen for DateECHODx)*
% ideally (if not available note as Normal, Mildly, Moderately, Severely decreased)	
RVEFMRI_Dx	RV ejection fraction as measurement for RV dysfunction on MRI *(on MRI chosen for DateMRI)*
% ideally (if not available note as Normal, Mildly, Moderately, Severely decreased)	
RVEFAngio_Dx	RV ejection fraction as measurement for RV dysfunction on RV angiogram *(on Angio chosen for DateAngioDx)*
% ideally (if not available note as Normal, Mildly, Moderately, Severely decreased)	
Exam_LVEF	Type of exam used for LVEF at diagnosis. MRI preferred over Echo and echo over Angio
1-MRI, 2-Echo, 3-Angio	
DateLVEF	Date of exam used for LVEF at diagnosis
dd-month-yyyy	

LVEF_Dx_Quant	Quantitative assessment of LVEF
%	
LVEF_Dx_Qual	Qualitative assessment of LVEF is quantitative not available
Normal, Mildly, Moderately, Severely decreased	

Manual imputation for RVEF:
1-RVEF on CMR is preferred for RVEF assessment.
2- For patients with assessment of RV function both with ultrasound and CMR:
We will compare the qualitative ultrasound value, establish the median value of MRI RVEF associated with each qualitative category (normal function, mild dysfunction, moderate dysfunction, severe dysfunction)
3- For patients with ultrasound-only assessed RV function, the median value calculated in step 2 will be assigned for the primary analysis.
Patients who only have a qualitative assessment of normal RVEF by MRI, will be assigned the median value of patients with normal MRI RV function (above 45%).

Manual imputation for LVEF:
1- LVEF on CMR is preferred for LVEF assessment.
2- If LVEF on CMR is not available, quantitative assessment by cardiac ultrasound will be used.
3- For patients with assessment of LV function both with ultrasound and MRI, we will compare the qualitative ultrasound value and establish the median value of MRI LVEF associated with each qualitative category (normal, mild dysfunction, moderate dysfunction, severe dysfunction).
4- For patients who only have a qualitative ultrasound assessment of LV function, the median value calculated in step 3 will be assigned

Task Force criteria at diagnosis
Cumulative: code the highest/most severe result a patient had for a specific test regardless of delay before dx and up to one year after dx and before the occurrence of the first event.

ImagingTFCDx	Reach of 2010 TFC Global or regional dysfunction and structural alterations imaging criteria (1 year before/after Dx and before the first event)
0=normal, 1=minor criteria, 2=major criteria	
RepolarizationTFCDx	Reach of 2010 TFC Repolarization criteria (1 year before/after Dx and before the first event)
0=normal, 1=minor criteria, 2=major criteria	
DepolarizationTFCDx	Reach of 2010 TFC Depolarization/conduction criteria (1 year before/after Dx and before the first event)
0=normal, 1=minor criteria, 2=major criteria	
ArrhythmiaTFCDx	Reach of 2010 TFC Arrhythmia criteria (1 year before/after Dx and before the first event)
0=normal, 1=minor criteria, 2=major criteria	
FamGenTFCDx	Reach of 2010 TFC Family history/Genetics criteria
0=normal, 1=minor criteria, 2=major criteria	
TissueTFCDx	Tissue characterization, according to 2010 TFC (1 year before/after Dx and before the first event)
0=normal, 1=minor criteria, 2=major criteria	
ICD	ICD implanted at any time
1=yes, 0=no	
Date_ICD implantation	Date of first ICD implantation
dd-month-yyyy	
ICDbaseline	ICD implanted any time before diagnosis up to one year after dx and before the first event.
ICD_MonitorZoneImplant	Cycle length of the Monitor zone at implant
milliseconds	
ICD_TxZone1Implant	Cycle length of the lowest therapy zone at implant
milliseconds	
ICD_MonitorZone_AryorEnd	Cycle length of the monitor zone at first LTVA or last programing available at follow-up
milliseconds	
ICD_Therapy_AryorEnd	Cycle length of the lowest therapy zone at first LTVA or last programing available at follow-up
milliseconds	

Medication history	
AAmedslistDx	List all cardiac anti-arrhythmic medication taken at diagnosis (list sotalol here)
0= none 1=Amiodarone 2=Sotalol 3=Class IC (Propafenone or Flecainide) 4=Dofetilide 5=Mexiletine 6= other	
BetablockersDx	Betablockers (excluding sotalol) taken at diagnosis
1=yes, 0=no	
AAmedslistEvent	List of all anti-arrhythmic medication taken at time of first event or censoring (list sotalol here)
0= none 1=Amiodarone 2=Sotalol 3=Class IC (Propafenone or Flecainide) 4=Dofetilide 5=Mexiletine 6= other	
BetablockersEvent	Betablockers (excluding sotalol) taken at time of first event or censoring
1=yes, 0=no	
OUTCOMES	
LTVAafterDx	Composite outcome of first ventricular arrhythmia
0 = no VT 1 = Spontaneous sustained VT **Definition:** *VT lasting ≥ 30 secs or with hemodynamic compromise at ≥ 100bpm or terminated by electrical cardioversion.* 2 = ICD intervention **Definition:** *ICD shock or antitachycardia overdrive pacing delivered in response to a ventricular tachyarrhythmia according to stored intracardiac ECG data.* 3 = SCA (aborted) **Definition:** *An event as described above, that is reversed, usually by cardiopulmonary resuscitation and/or defibrillation or cardioversion.* 4 = SCD **Definition:** *Death of cardiac origin that occurred unexpectedly within 1 hour of the onset of new symptoms or a death that was unwitnessed and unexpected.*	
DateLTVAafterDx	Date of 1st composite outcome of first life threatening ventricular arrhythmia
dd-month-yyyy	
LTVAafterDx_CL	Cycle length of ventricular arrhythmia coded for primary outcome
Milliseconds	
SevereLTVAafterDx	VT with CL≤ 240 ms(≥250 bpm), FV, SCD or resuscitated SCD
0 = no VT 1 = Spontanous sustained VT CL≤ 240 ms (≥ 250 bpm) **Definition:** *VT (*CL≤ 240 ms (≥ 250 bpm) *lasting ≥ 30 secs or with hemodynamic compromise at ≥ 100bpm or terminated by electrical cardioversion.* 2 = ICD intervention for VT CL≤ 240 ms (≥ 250 bpm*)* **Definition:** *ICD shock or antitachycardia overdrive pacing delivered in response to a ventricular tachyarrhythmia according to stored intracardiac ECG data.* 3 = SCA (aborted): **Definition:** *An event as described above, that is reversed, usually by cardiopulmonary resuscitation and/or defibrillation or cardioversion.* 4 = SCD **Definition:** *Death of cardiac origin that occurred unexpectedly within 1 hour of the onset of new symptoms or a death that was unwitnessed and unexpected.*	
DateSevereLTVAafterDx	Date of 1st Severe LTVA (VT with CL≤ 240 ms (≥250 bpm) or VF, SCD or resuscitated SCD)
dd-month-yyyy	
SevereLTVAafterDx_CL	Cycle length of severe LTVA
milliseconds	
Transplant	Cardiac transplant at follow-up
1=yes, 0=no	
Date_Transplant	Date of cardiac transplant
dd-month-yyyy	
Death	Death during follow-up
1=yes, 0=no	
DateOFdeath	Date of death
dd-month-yyyy	
CauseDeath_text	Cause of death
text	
CauseDeath_cat	Cause of death categorized
1=SCD, 2=heart failure, 3=arrhythmic and heart failure (eg. heart failure largely caused by arrhythmias, 4= non-cardiac	
Other variables at follow-up	

VTAblation	Endocardial or epicardial VT ablation performed at any time before last coded event
1=yes, 0=no	
DateVTAblation	Date of first ablation
dd-month-yyyy	
AtrialArrhythmia	Date of first documented sustained atrial arrhythmia of more than 30 seconds
	0=No 1=Atrial fibrillation 2=Atrial Flutter 3-Atrial Tachycardia
Date_AtrialArrhythmia	Date of 1st documented sustained atrial arrhythmia
	dd-month-yyyy
AdditionalNotes	Additional information about the patient if necessary
text	
DateLFU	Date of last clinical follow-up allowing assertion of outcomes: Censoring or last event coded for outcome
dd-month-yyyy	
Adjudication_outcome	Is adjudication for outcomes possible for both arrhythmic outcomes
Text	

Table S3. Baseline characteristics according to genotype

	Negative genotype (n=84)	*PKP2* (n=111)	*DSP* (n=38)	*DSG2* (n=27)	*DSC2* (n=3)	*TMEM43* (n=10)	*PLN* (n=3)	Multiple mutations (n=6)	p-value
Total (282 tested)	84(29.8)	111(39.3)	38(13.5)	27(9.6)	3(1.1)	10(3.5)	3(1.1)	6(2.1)	
Demographics									
Age at diagnosis (years)	45.0±16.1	40.1±15.5	38.9±15.3	37.9±15.2	51.7±14.6	42.0±15.9	43.3±8.0	36.4±14.4	0.219
Male sex	50 (59.5)	54 (49.1)	11 (28.9)	9 (33.3)	1 (33.3)	6 (60.0)	0	3 (50.0)	0.025
Proband status	63 (75.0)	47 (42.7)	27(71.1)	16(59.3)	2 (66.7)	2 (20.0)	2 (66.7)	4 (66.7)	<0.001
History									
Recent cardiac syncope (n=424)	8 (9.5)	9 (8.2)	4 (10.5)	2 (7.4)	0	0	1 (33.3)	1 (16.7)	0.727
ECG/continuous ECG monitoring									
TWI in ≥ 3 precordial leads	50 (59.5)	68 (61.8)	18 (47.4)	22 (81.5)	1 (33.3)	1 (10.0)	2 (66.7)	2 (33.3)	0.003
TWI in ≥ 2 inferior leads	24 (28.6)	28 (25.5)	6 (15.8)	9 (33.3)	1 (33.3)	1 (10.0)	1 (33.3)	2 (33.3)	0.216
PVC count (n=324)	1779 [518-6000]	871 [163-2365]	1524 [386-4599]	925 [615-2450]	2100 [1959-2188]	5723 [1301-10600]	5968 [3242-14973]	2650 [2108-2927]	0.159
NSVT (n=359)	32 (38.1)	36 (32.7)	18 (47.4)	6 (22.2)	2 (66.7)	1 (10.0)	2 (66.7)	3 (50.0)	0.138
Imaging									
RVEF (%) (n=410)	43 [35- 53]	48 [39- 53]	49 [42-54]	40 [28-52]	65 [53- 68]	48 [43- 50]	48 [43- 51]	38 [35- 42]	0.054
LVEF (%) (n=404)	55 [50- 60]	58 [55- 62]	50 [43- 58]	58 [50- 60]	55 [40- 61]	55 [40- 58]	50 [47- 54]	60 [58- 62]	<0.001
LVEF <50% (n=404)	17 (20.2)	7 (6.4)	17 (44.7)	4 (14.8)	1 (33.3)	4 (40.0)	1 (33.3)	1 (16.7)	0.002
Treatment at baseline									
ICD	40 (40.7)	34 (30.9)	12 (31.6)	7 (25.9)	3 (100.0)	7 (70.0)	0 (0.0)	6 (100.0)	<0.001
Anti-arrhythmic drugs (n=408)									
Amiodarone	68 (81)	79 (71.8)	34 (89.5)	22 (81.5)	3 (100)	8 (80.0)	2 (66.7)	6 (100.0)	NA
Sotalol	8 (9.5)	23 (20.9)	3 (7.9)	3 (11.1)	0	2 (20.0)	1 (33.3)	0	
Propafenone/ Flecainide	3 (3.6)	3 (2.7)	1 (2.6)	1 (3.7)	0	0	0	0	

β-blockers (n=407)	43 (51.2)	43 (39.1)	28 (73.7)	10 (37.0)	3 (100.0)	1 (10.0)	1 (33.3)	4 (66.7)	0.012
VA events	16(19)	27(24.5)	5(13.2)	5(18.5)	2(66.7)	6(60.0)	0(0)	3(50.0)	0.013
Transplant	1 (1.2)	1 (0.9)	0	2 (7.4)	0	0	0	1 (16.7)	NA
Death	1 (1.2)	1 (0.9)	1 (2.6)	0	0	0	0	0	0.984
Follow-up	4.51 [1.76-6.42]	3.65 [1.88-7.42]	2.99 [1.15-6.50]	3.20 [1.15-7.29]	8.79 [7.81-10.08]	6.46 [5.12-9.75]	3.64 [2.52-7.48]	2.99 [2.44-3.89]	0.475

Table S4: Baseline Characteristics by Country

	Overall (n=429)	**Canada (n=79**	**Denmark (n=27)**	**France (n=52)**	**Italy (n=176)**	**Spain (n=49)**	**USA (n=46)**	**P-value**
Demographics								
Age at diagnosis (years)	43.12 (±15.82)	41.61 (±15.87)	44.02 (±17.58)	41.41 (±15.72)	45.42 (±14.97)	43.59 (±16.95)	37.88 (±15.79)	0.068
Male sex	235 (54.8)	40 (50.6)	15 (55.6)	30 (57.7)	115 (65.3)	23 (46.9)	12 (26.1)	<0.001
Proband status	278 (64.8)	40 (49.4)	17 (37.0)	10 (80.8)	36 (79.5)	26 (46.9)	22 (52.2)	<0.001
Pathogenic mutation (n= 281)								<0.001
PKP2	111 (25.6)	23 (29.1)	7 (25.9)	16 (15.4)	27 (15.3)	19 (38.8)	18 (34.6)	
DSP	38 (8.9)	3 (3.8)	2(7.4)	18(34.6)	14 (8.0)	18(36.7)	12(26.1)	
DSG2	27 (6.3)	3 (3.8)	11 (40.7)	3 (5.8)	2 (1.1)	4 (8.2)	2 (4.3)	
DSC2	3 (0.7)	2 (2.5)	0 (0.0)	6 (11.5)	1 (0.6)	3 (6.1)	0 (0.0)	
JUP	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
TMEM43	10 (2.3)	7 (8.9)	1 (3.7)	0 (0.0)	2 (1.1)	0 (0.0)	1 (3.7)	
PLN	3 (0.7)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Multiple mutations	6 (1.4)	3 (3.8)	2 (7.4)	1 (1.9)	0 (0.0)	0 (0.0)	2 (7.4)	
History								
Cardiac syncope (n=424)	37 (8.6)	5 (6.3)	3 (11.1)	10(19.2)	10 (5.7)	6 (12.2)	3 (6.5)	0.087
ECG/continuous ECG monitoring								
TWI in ≥ 3 precordial leads	250 (58.3)	34 (43.0)	26 (56.5)	115 (65.3)	18 (66.7)	29 (59.2)	28 (53.8)	0.007
TWI in ≥ 2 inferior leads	109 (25.4)	17 (21.5)	6 (13.0)	57 (32.4)	6 (22.2)	10 (20.4)	13 (25.0)	0.002

PVC count (n=324)	1434.00 [438.75, 3600.75]	722.00 [37.00, 3308.00]	1800.00 [800.00, 2800.00]	1551.00 [401.00, 3158.00]	1500.00 [570.00, 4147.00]	1310.00 [556.00, 3634.50]	1044.00 [192.50, 3276.00]	0.157
NSVT (%) (n=359)	148 (34.5)	22 (27.8)	7 (25.9)	21 (40.4)	67 (38.1)	10 (20.4)	21 (45.7)	<0.001
Imaging								
RVEF (%) (n=410)	45.00 [36.00, 53.00]	41.00 [35.00, 48.50]	48.50 [30.00, 53.00]	40.00 [30.00, 48.00]	48.25 [40.00, 55.00]	43.50 [33.25, 52.00]	47.00 [39.00, 52.00]	<0.001
LVEF (%) (n=404)	57.00 [51.00, 60.00]	55.00 [52.00, 58.00]	58.00 [53.00, 60.00]	57.00 [52.00, 61.75]	57.00 [50.00, 60.00]	58.00 [52.25, 60.75]	57.50 [52.00, 62.00]	0.591
LVEF <50 (%)(n=404)		14(17.7)	2 (7.4)	7 (13.5)	38(21.6)	7 (14.3)	8 (17.4)	0.555
Treatment at baseline								
ICD	175(40.8)	47(59.5)	14 (51.9)	6 (11.5)	50(28.4)	15(30.6)	21(45.7)	<0.001
Anti-arrhythmic drugs (n=408)								<0.001
Amiodarone	23 (6)	1 (1.3)	1 (3.7)	0 (0.0)	20(11.5)	0 (0.0)	4 (8.7)	
Sotalol	79 (18.4)	3 (3.8)	0(0.0)	9(17.3)	48(27.3)	16(32.7)	3 (6.5)	
Propafenone/ Flecainide	15 (3.5)	0 (0.0)	0 (0.0)	4 (7.7)	11 (6.2)	0 (0.0)	0 (0.0)	
β-blockers (%) (n=407)	206 (48.0)	47 (59.5)	8 (29.6)	24 (46.2)	94 (53.4)	13 (26.5)	20 (43.5)	<0.001
Transplant (%)	0 (0.0)	0 (0.0)	2 (4.1)	0 (0.0)	3 (11.1)	1 (1.9)	0 (0.0)	<0.001
Death	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	2 (7.4)	1 (1.9)	5 (2.8)	0.231
Follow-up	5.02 [2.05, 7.90]	5.31 [2.43, 7.88]	2.05 [1.03, 6.11]	3.56 [1.41, 7.50]	5.45 [3.05, 9.15]	5.64 [3.10, 10.88]	1.28 [0.65, 2.74]	<0.001

Variables are expressed as frequency (%), mean ± standard deviation, or median [IQR]. Total number of patients for a given variable mentioned if missing data. DSG2, desmoglein-2; DSP, desmoplakin; ICD, implantable cardioverter-defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; NSVT, non-sustained ventricular tachycardia; JUP, junction plakoglobin; PKP2, plakophilin-2; PLN, phospholamban; PVC, premature ventricular complex; RVEF, right ventricular ejection fraction; TMEM4, Transmembrane Protein 43; TWI, T-wave inversion; VA, ventricular arrhythmia

Table S5: Baseline clinical characteristics of the derivation and validation cohorts

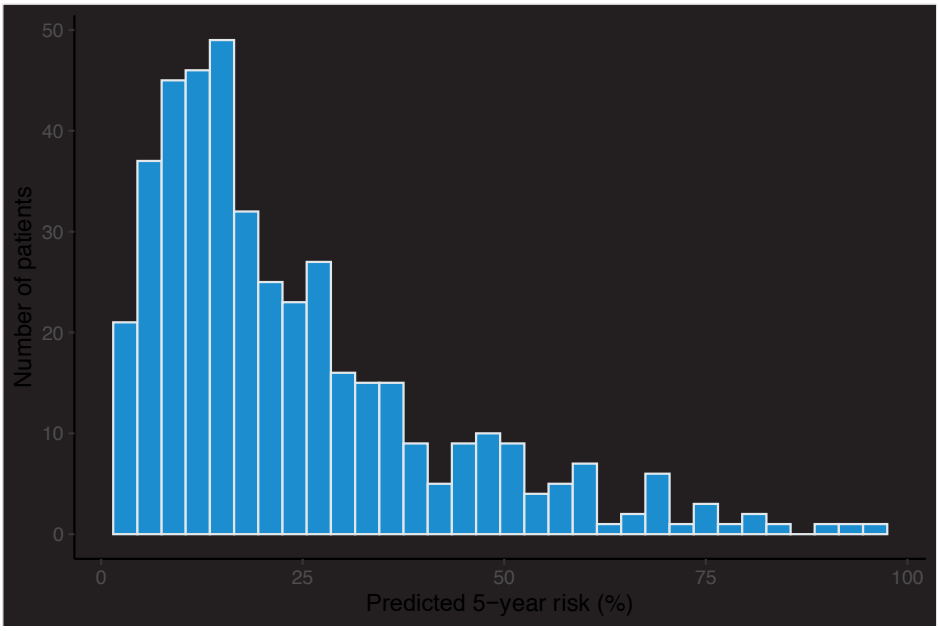
	Overall (n=957)	Derivation cohort (n=528)	Validation cohort (n=429)	P-value
Demographics				
Age at diagnosis (years)	40.4 (±15.81)	38.2 (±15.47)	43.1 (±15.82)	<0.001
Male sex	471 (49.2)	236 (44.7)	235 (54.8)	0.002
Proband status	541 (56.5)	263 (49.8)	278 (64.8)	<0.001
Pathogenic mutation (n= 683)				<0.001
PKP2	370 (38.7)	260 (49.2)	111 (25.6)	
DSP	61 (6.4)	23 (4.4)	38 (8.9)	
DSG2	47 (4.9)	20 (3.8)	27 (6.3)	
DSC2	10 (1.0)	7 (1.3)	3 (0.7)	
JUP	1 (0.1)	1 (0.2)	0 (0.0)	
TMEM43	11 (1.1)	1 (0.2)	10 (2.3)	
PLN	30 (3.1)	27 (5.1)	3 (0.7)	
Multiple mutations	19 (2.0)	13 (2.5)	6 (1.4)	
History				
Cardiac syncope (n=952)	105 (11.0)	68 (12.9)	37 (8.6)	0.006
Recent cardiac syncope (n=952)	89 (9.3)	57 (10.8)	32 (7.5)	0.011
ECG/continuous ECG monitoring				
TWI in ≥ 3 precordial leads	548 (57.3)	298 (56.4)	250 (58.3)	0.046
TWI in ≥ 2 inferior leads	194 (20.3)	85 (16.1)	109 (25.4)	<0.001
PVC count (n=749)	1115.00 [357.00, 3686.00]	1007.00 [278.00, 3731.00]	1434.00 [438.75, 3600.75]	0.228
NSVT (%) (n=829)	379 (39.6)	231 (43.8)	148 (34.5)	0.004
Imaging				
RVEF (%) (n=919)	46.73 [37.00, 51.00]	48.00 [38.00, 50.79]	45.00 [36.00, 53.00]	0.641
LVEF (%) (n=919)	58.00 [53.00, 62.00]	60.00 [54.17, 62.00]	57.00 [51.00, 60.00]	<0.001
LVEF < 50% (n=919)	143 (14.9)	67 (12.7)	76 (17.7)	0.002
Treatment at baseline				
ICD	392 (41.0)	217 (41.1)	175 (40.8)	0.976
Anti-arrhythmic drugs (n=918)				<0.001
Amiodarone	32 (3.3)	9 (1.7)	23 (5.4)	
Sotalol	131 (13.7)	52 (9.8)	79 (18.4)	

Propafenone/ Flecainide	19 (2.0)	4 (0.8)	15 (3.5)	
Betablockers (%) (n=918)	406 (42.4)	200 (37.9)	206 (48.0)	0.001
Transplant (%) (n=951)	90 (9.4)	54 (10.2)	36 (8.4)	<0.001
Death (%)	27 (2.8)	18 (3.4)	9 (2.1)	0.307
Follow-up	4.97 [2.23, 8.88]	4.83 [2.44, 9.33]	5.02 [2.05, 7.90]	0.083

Variables are expressed as frequency (%), mean ± standard deviation, or median [IQR]. Total number of patients for a given variable mentioned if missing data. DSG2, desmoglein-2; DSP, desmoplakin; ICD, implantable cardioverter-defibrillator; IQR, interquartile range; LVEF, left ventricular ejection fraction; NSVT, non-sustained ventricular tachycardia; JUP, junction plakoglobin; PKP2, plakophilin-2; PLN, phospholamban; PVC, premature ventricular complex; RVEF, right ventricular ejection fraction; TMEM43, Transmembrane Protein 43; TWI, T-wave inversion; VA, ventricular arrhythmia.

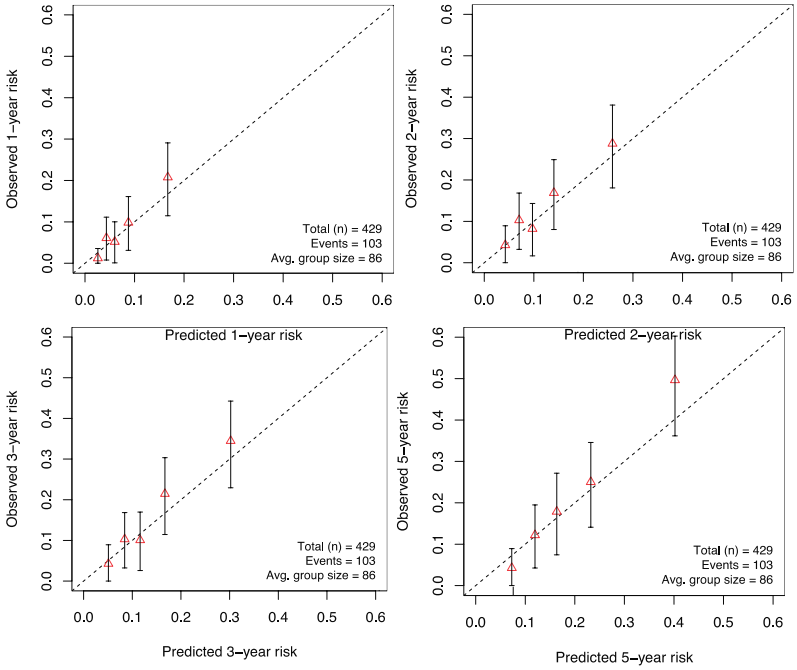
Supplementary Figures

Supplementary figure S1 Distribution of predicted risk in the current (validation) cohort.



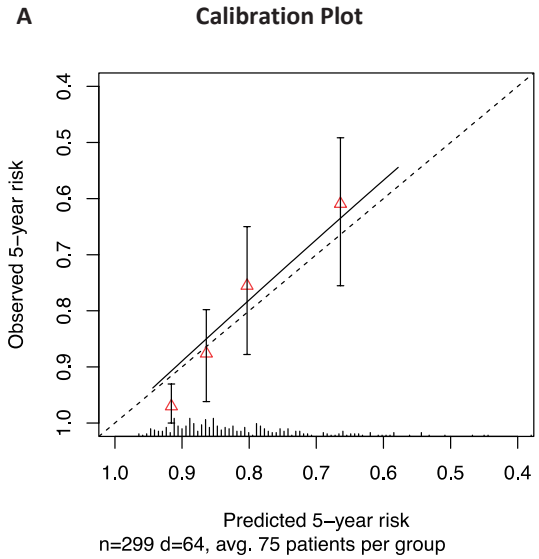
The number of patients (y axis) is presented for each predicted risk according to the model (x axis).

Supplementary figure S2 Calibration plots presenting the agreement between predicted (x axis) and observed (y axis) at 1-year, 2 years, 3 years and 5-year risk of ventricular arrhythmia (VA).



Triangles represent binned Kaplan–Meier estimates with 95% confidence intervals for quintiles of predicted risk. The calibration is shown to be acceptable across the risk spectrum with no significant under or over prediction in any risk category. VA, ventricular arrhythmia.

Supplementary figure S3: Complete case analysis

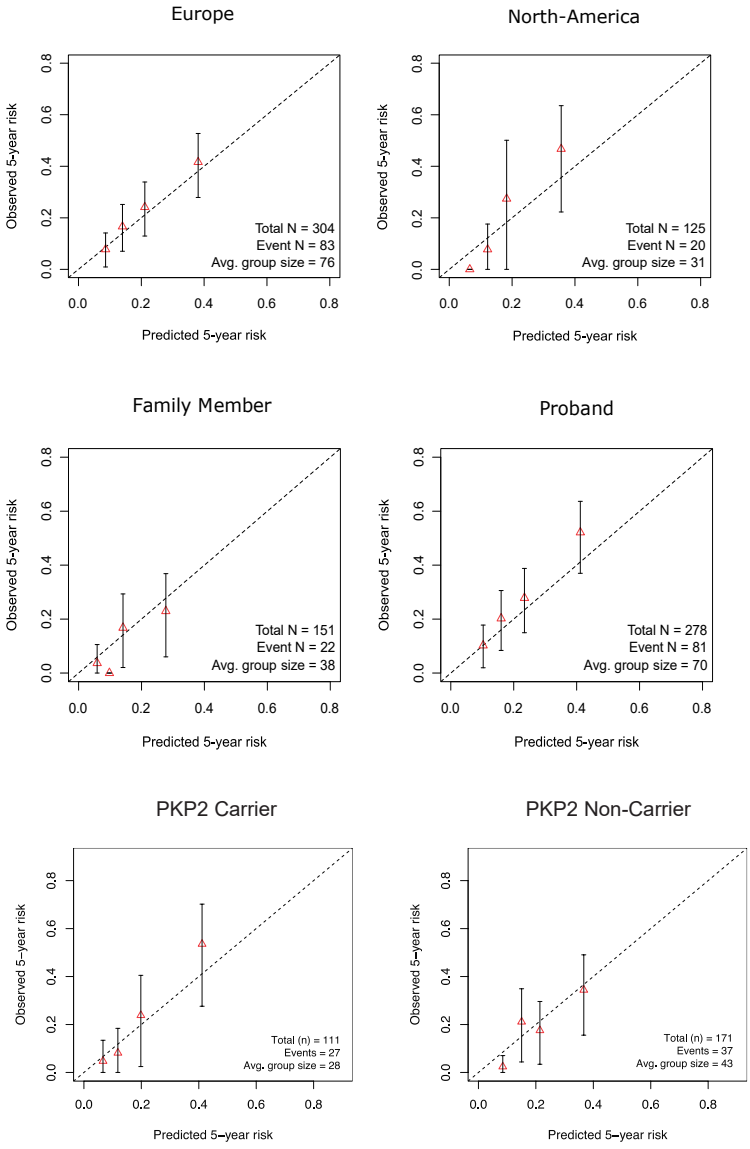


	Performance Parameters	
	Value	95 CI
C-index	0.68	0.61-0.75
Calibration slope	1.02	0.99-1.06

	Probability of survival	
Time	Derivation cohort	Validation cohort (Complete case)
1 year	0.938	0.933
2 years	0.901	0.906
3 years	0.880	0.890
4 years	0.870	0.849
5 years	0.840	0.834

299 patients had complete data. The calibration plot shows an acceptable concordance between predictions and observations with a possible overestimation in the low-risk patients. Calibration plot is described in supplementary table S2. C-index and Calibration slope are comparable to the results obtained in the complete cohort.

Supplementary Figure S4. Calibration according to different subgroups: geographic origin, pedigree information and Plakophilin 2 variant carrier.

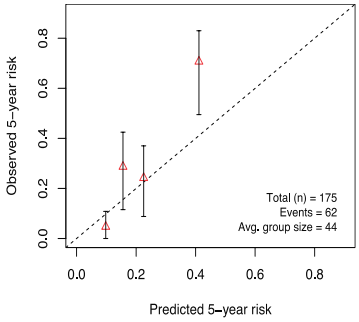


Calibration plot showing the agreement between predicted (X-axis) and observed (Y-axis) 5 year risk of developing life-threatening ventricular arrhythmia in patients from North America vs Europe, Family members vs Probands, Plakophilin 2 (likely) pathogenic variants carriers and without

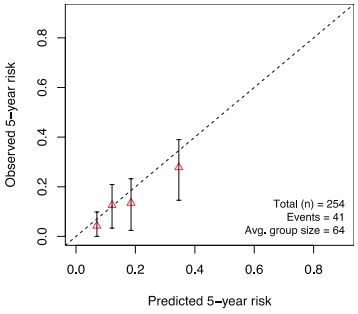
Plakophilin 2 (likely) pathogenic variants. Triangles represent binned Kaplan-Meier estimates with 95% confidence intervals for quintiles of predicted risk. Dotted line represents perfect calibration with observation directly corresponding to the predictions. Spike histogram on the X-axis reflects the number of patients with a predicted risk corresponding to the X-axis value.

Supplementary Figure S5. Calibration according to implantable cardioverter-defibrillator carrier (ICD) status

Prophylactic ICD carrier



Prophylactic ICD non carrier



Calibration plot showing the agreement between predicted (X-axis) and observed (Y-axis) 5 year risk of developing life-threatening ventricular arrhythmia in patients with and without ICDs at baseline, defined as one year after to diagnosis and prior to first event. Description of the plot is as per *Supplementary Figure S3*

PART II

IMPROVING THE ARVC RISK CALCULATOR

CHAPTER 6

PROGRAMMED VENTRICULAR STIMULATION AS AN ADDITIONAL PRIMARY PREVENTION RISK STRATIFICATION TOOL IN ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY: A MULTINATIONAL STUDY

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Circulation. *2022 Nov 8;146(19):1434-1443. doi: 10.1161/CIRCULATIONAHA.122.060866. PMID: 36205131*

ABSTRACT

Background: A novel risk calculator based on clinical characteristics and non-invasive tests that predicts the onset of clinical sustained ventricular arrhythmias (VA) in patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) has been proposed and validated by recent studies. It remains unknown whether programmed ventricular stimulation (PVS) provides additional prognostic value.

Methods: All patients with a definite ARVC diagnosis, no history of sustained VAs at diagnosis, and PVS performed at baseline were extracted from 6 international ARVC registries. The calculator-predicted risk for sustained VA [sustained or implantable cardioverter-defibrillator treated ventricular tachycardia (VT) or fibrillation, (aborted) sudden cardiac arrest] was assessed in all patients. Independent and combined performance of the risk calculator and PVS on sustained VA were assessed during a 5-year follow-up period.

Results: Two-hundred and eighty-eight patients (41.0±14.5 years, 55.9% male, right ventricular ejection fraction 42.5±11.1%) were enrolled. At PVS, 137 (47.6%) patients had inducible VT. During a median of 5.31 [2.89–10.17] years of follow-up, 83 (60.6%) patients with a positive PVS and 37 (24.5%) with a negative PVS experienced sustained VA ($p<0.001$). Inducible VT predicted clinical sustained VA during the 5-year follow-up and remained an independent predictor after accounting for the calculator-predicted risk (HR 2.52 [1.58–4.02]; $p <0.001$). Compared to ARVC risk calculator predictions in isolation (C-statistic 0.72), addition of PVS inducibility showed improved prediction of VA events (C-statistic 0.75) (LLR for nested models: $p<0.001$). PVS inducibility had a 76 [67–84]% sensitivity and 68 [61–74]% specificity, corresponding to log-likelihood ratios (LR) of 2.3 and 0.36 for inducible (LR+) and non-inducible (LR-) patients, respectively. In patients with a ARVC risk calculator-predicted risk of clinical VA events <25% over 5-years (i.e., low/intermediate subgroup), PVS had a 92.6% negative predictive value (NPV).

Conclusion: PVS significantly improved risk stratification above and beyond the calculator-predicted risk of VA in a primary prevention cohort of patients with ARVC, mainly for patients considered to be at low risk by the clinical risk-calculator.

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a cardiomyopathy characterized by progressive cardiomyocyte loss and fibro-fatty replacement.[1] Patients with ARVC are at risk for life-threatening ventricular arrhythmias (VA) and sudden cardiac death (SCD), which may even represent the first clinical manifestation of the disease.[1,2]

The placement of an implantable cardioverter defibrillator (ICD) is a crucial component of ARVC management.[2,3] Nonetheless, arrhythmic risk stratification and the selection of the optimal candidates for ICD placement, especially for primary prevention of SCD, has proven difficult.[4] A VA risk calculator in patients without previous sustained VAs has recently proposed.[5] This risk calculator included seven clinical variables derived from non-invasive tests that are routinely performed in ARVC patients. Its utility has been replicated in independent cohorts and it has been shown to perform better than risk stratification algorithms currently proposed by consensus statements.[6–12] Since its publication, the possibility of integrating additional parameters such as ventricular tachycardia (VT) inducibility on programmed ventricular stimulation (PVS) with the risk calculator has been suggested.[13]

The role of PVS for arrhythmic risk stratification in primary prevention ARVC has been debated. Although some studies supported its role as a predictor of sustained VA[14–19] others have reported a poor positive predictive value.[20] Currently available studies, however, suffered from a relatively limited sample size and often grouped together primary and secondary prevention ARVC patients. The aim of this study was to investigate in a large multicenter cohort of patients with ARVC, whether PVS has prognostic value independent of the existing ARVC VA risk calculator in order to further improve primary prevention arrhythmic risk stratification.

METHODS

Patient Population

We conducted an observational, retrospective, multicenter cohort study.

The study population was extracted from six ARVC registries at academic institutions in seven countries across North America and Europe. From each registry, all patients who met the following inclusion criteria were included in the current study: 1) Diagnosed with definite ARVC as per the 2010 Task Force Criteria (TFC);[21] 2) Absence of spontaneous sustained VA or aborted SCD at disease diagnosis; 3) performance of a PVS within one year before to one year after disease diagnosis, and prior to any sustained VA or SCD event.

The study was conducted in accordance to the declaration of Helsinki and was approved by local ethics and/or institutional review boards and consent was obtained in accordance with national requirements. To maintain patient confidentiality, data and study materials will not be made available to other researchers for purposes of replicating the results. A limited dataset may be made available upon request.

Variables and Outcomes Definition

For each patient, baseline demographic variables, data from ECG, echocardiography, cardiac magnetic resonance (CMR), and all seven variables [age, sex, syncope of clear cardiac origin, number of leads with T wave inversion on a 12-lead ECG (sum of anterior and inferior leads; TWI), Non-sustained ventricular tachycardia (NSVT), 24-hour premature ventricular complex (PVC) count, right ventricular ejection fraction (RVEF)] included in the ARVC risk calculator (ARVCrisk.com) were collected independently by each registry, in accordance to standard operating procedures and definitions previously presented.[5] All genetic variants reported were adjudicated according to the 2015 American College of Medical Genetics and Genomics guidelines.[22]

PVS data were collected by study report, direct tracing, or medical record review. For each study, data regarding the stimulation protocol, the cycle length and the morphology of all different VAs induced during PVS, and the baseline conduction measurements (atrial-His and His-ventricular time) were collected. A positive PVS was defined as the induction of a sustained monomorphic VT lasting ≥30 s or leading to hemodynamic compromise. Patients were accordingly classified into inducible (PVS+ group) and a non-inducible (PVS- group) groups.

Sustained VA was defined as a composite of SCD, sustained VT (lasting ≥30 s or with hemodynamic compromise or requiring cardioversion), ventricular fibrillation/flutter (VF), or appropriate ICD intervention as reported previously.[5] Rapid VA/(aborted) SCD were defined as sustained VT ≥ 250 bpm (cycle length ≤ 240 ms), VF, SCD or aborted SCD.[5] Sustained VA were assessed using a combination of the ECG tracings, Holter ECG results, ICD interrogations, and clinical reports available at follow-up, as collected per each registry practice.

The primary outcome of the study was the comparison of rates of first sustained VA within five years after disease diagnosis by the 2010 TFC between patients with positive and negative PVS. The rates of first episode of rapid VA/(aborted)SCD as well as heart transplant, cardiovascular, and all-cause mortality were also assessed and compared in the two groups.

Statistical Analyses

Of important note, a correction of the risk calculator's baseline survival was issued after its original publication. This manuscript is based on the corrected version[23]. Continuous variables were expressed using mean ± standard deviation (s.d.) or median [interquartile range (IQR)], and comparisons were performed using an independent sample Student's t-test or Mann-Whitney U-test, in accordance to their distribution. Categorical variables were reported as counts (percentage) and comparisons run using χ^2 or Fisher's exact tests, as appropriate. The association between baseline characteristics and PVS inducibility status was tested using univariable logistic regression; those variables that met a significance threshold of 0.10 were included in a multivariable logistic

regression model. Association between cycle length of VT induced during PVS and of VA observed during follow-up was tested using linear regression, with strength of association determined using the Pearson correlation coefficient. Rates of VA-free survival were assessed using Kaplan-Meier curves, and compared using log-rank testing. The risk of sustained VA at 5 years was predicted for each patient using the ARVC risk calculator (ARVCrisk.com) and calculated according to **Equation1**, where PI is the prognostic index and is calculated according to **Equation2**.

$$\text{(1)} \qquad \text{5-year VA Risk} = 1 - 0.840 * \exp(PI)$$

$$\text{(2)} \qquad PI = Sex * 0.49 - Age * 0.022 + Cardiac\ Syncope * 0.66 + NSVT * 0.81 + \ln(24hr\ PVC\ count) * 0.17 + TWI * 0.11 - RVEF * 0.025$$

Model calibration was assessed visually using a plot of predicted versus observed event rates. All covariates of the ARVC risk calculator had < 5% missingness, except for NSVT (6% missingness), 24-hr PVC count (11% missingness), and RVEF (12% missingness). Missing quantitative values for RVEF and left ventricular ejection fraction (LVEF) were imputed manually when qualitative assessment was present, in accordance to previously described methods[5]. Other missing data used for the ARVC risk calculator were assumed to be missing at random and imputed using multiple imputations with chained equations[24]. Complete-case sensitivity analysis was performed to test the impact of this imputation.

To assess the predictive ability of PVS inducibility for VA events, Cox proportional hazards models of VA events were fitted to the result of PVS testing, both as a single coefficient and in conjunction with the ARVC risk calculator PI (incorporated as a fixed offset variable). Model discriminations were assessed using a non-parametric concordance-based C-statistic[25]. Both PVS inducibility and the individual coefficients of the ARVC risk calculator fulfilled standard proportional hazards assumption testing criteria. The added value of PVS inducibility to the ARVC risk calculator for predicting VA events was assessed using log-likelihood ratio (LLR) testing for nested models, with 1 degree-of-freedom added to the 7 degrees-of-freedom of the original ARVC risk calculator. Net reclassification improvement for a 5-year risk cut-off of 25% (5% risk/year) was also calculated

using standard approximations for time-to-event data[26]. Patients were then stratified by ARVC risk calculator predicted risk into 'low/intermediate arrhythmic risk' (<5% predicted risk/year; <25% predicted risk over 5 years) and 'high arrhythmic risk' (>=5% predicted risk/year; >=25% predicted risk over 5 years) sub-cohorts. The sensitivity and specificity of PVS inducibility in the overall cohort, and the positive predictive value (PPV) and negative predictive value (NPV) of PVS inducibility in these 2 subgroups were calculated according to previously published methods for estimating these test metrics in survival data[27]. The sensitivity and specificity were then used to determine the impact of the use of PVS in addition to the risk calculator in a given patient using the Bayes' theorem following these sequential equations (**Equations 3, 4, 5**):

$$(3) \qquad Pre\text{-}test\ Odds = \frac{ARVC\ risk\ prediction}{1-ARVC\ risk\ prediction}$$

$$(4) \qquad Post\text{-}test\ Odds = Pre\text{-}test\ Odds * LR$$

$$(5) \qquad Updated\ VA\ Risk = \frac{Post\ PVS\ Odds}{1+Post\ PVS\ Odds}$$

Here, LR is the likelihood ratio and is calculated using Equation 6 for inducible PVS (+LR) and Equation 7 for non-inducible PVS (-LR).

$$(6) \qquad +LR = \frac{Sensitivity}{(1-Specificity)}$$

$$(7) \qquad -LR = \frac{(1-Sensitivity)}{Specificity}$$

All analyses were performed using STATA (v.14.0 STATA Corp, College Station, Lake Street Way, TX, USA), PyCharm (v 2018.3.6 Community Edition, JetBrains Inc., Boston, MA, USA), and the python Lifelines and statsmodels statistical software package. For all statistical testing, $p<0.05$ was used as a threshold for significance.

RESULTS

Overall cohort

Two-hundred and eighty-eight definite ARVC patients from 12 institutions in 7 countries who underwent PVS at the time of diagnosis were included in the study. The mean age at diagnosis was 41.0±14.5 years, 55.9% of the patients were male and 73.6% were probands. Genetic testing was performed in 243 patients (84.4%), 141 (58.0%) of whom harbored ARVC associated pathogenic or likely pathogenic variants. Variants were most common in *PKP2* (n=96), followed by *DSP* (n=11) and *PLN* (n=11). Overall characteristics of the study cohort are reported in **Table1**.

Table 1. Cohort characteristics

	Overall (n=288)	PVS+ group (n=137)	PVS- group (n=151)	p
Age (years), mean±s.d.	41.0±14.5	39.2±14.0	42.7±14.7	**0.037**
Male sex, n (%)	161 (55.9)	82 (59.9)	79 (52.3)	0.198
European ancestry / White, n (%)	277 (98.2)	136 (99.3)	141 (97.2)	0.413
Proband Status, n (%)	212 (73.6)	111 (81.0)	101 (66.9)	**0.007**
Pathogenic/likely pathogenic variant (genetic available n=243)				
PKP2, n (%)	96 (39.5)	54 (44.3)	42 (34.7)	
DSP, n (%)	11 (4.5)	4 (3.3)	7 (5.8)	0.713
DSG2, n (%)	9 (3.7)	5 (4.1)	4 (3.3)	
PLN, n (%)	11 (4.5)	3 (2.5)	8 (6.6)	
Other, n (%)	14 (5.8)	5 (4.1)	9 (7.4)	
Recent cardiac syncope, n (%)	69 (24.0)	38 (27.7)	31 (20.5)	0.152
Number of leads with TWI, median [IQR]	4 [3–5]	4 [3–5]	3 [2–5]	**0.003**
NSVT at diagnosis, n (%)	134 (46.5)	77 (56.2)	57 (37.8)	**0.002**
24-h PVC count, median (n= 235) [IQR]	1445 [500–3731]	1624 [600–4630]	1154 [475–2788]	**0.026**
CMR at baseline (n=264)				
RVEF (%), mean ± s.d.	42.5±11.1	40.8±11.4	43.9±10.6	**0.027**
LVEF (%), mean ± s.d.	55.7±8.7	55.5±9.3	55.8±8.2	0.802
Treatment at baseline,				
Beta-blockers, n (%)	123 (42.7)	63 (46.0)	60 (39.7)	0.284
Anti-arrhythmic drugs, n (%)	102 (35.4)	46 (33.6)	56 (37.1)	0.534
ICD at disease diagnosis, n (%)	78 (27.1)	53 (38.7)	25 (16.6)	**<0.001**

CMR: cardiac magnetic resonance; DSG2:desmoglein-2; DSP: desmoplakin; ICD: implantable cardioverter defibrillator; LVEF: left ventricular ejection fraction; NSVT: non-sustained ventricular tachycardia; PKP2: plakophilin-2; PLN: phospholamban PVC: premature ventricular contraction; PVS: programmed ventricular stimulation; RVEF: right ventricular ejection fraction; TWI: T wave inversion.

One-hundred and ninety-nine (69.1%) patients were part of the ARVC risk calculator development cohort[5] while 89 (31%) additional patients were derived from an Italian cohort in which the risk calculator has previously been validated.[6] Comparison of the study cohort with the ARVC risk calculator development cohort is reported in **Table2**. Patient characteristics per registry have been reported in **Section C** of the Supplementary Material.

Table 2. Comparison of the baseline characteristics of the study cohort and of the cohort used for the ARVC risk calculator derivation

	Study cohort (n=288)	ARVC risk calculator cohort[5] (n=528)	p
Age, mean±s.d.	41.0±14.5	38.16±15.47	**0.011**
Male sex, n (%)	161 (55.9)	236 (44.7)	**0.002**
Caucasian, n (%)	277 (98.2)	485 (91.9)	**<0.001**
Proband Status, n (%)	212 (73.6)	263 (49.8)	**<0.001**
Genetic test available, n (%)	243 (84.4)	504 (95.4)	**<0.001**
Pathogenic variant, n (%)	141 (58.0)	340 (67.5)	**<0.001**
PKP2, n (%)	96 (39.5)	258 (51.2)	**<0.001**
Non *PKP2*, n (%)	45 (18.5)	82 (16.3)	0.636
Recent cardiac syncope, n (%)	69 (24.0)	48 (9.1)	**<0.001**
Leads with TWI on ECG			
TWI in ≥3 precordial leads, n (%)	189 (65.6)	298 (56.4)	**0.016**
TWI in ≥2 inferior leads, n (%)	71 (24.7)	85 (16.1)	**0.003**
NSVT at diagnosis, n (%)	134 (46.5)	231 (43.8)	0.446
24-h PVC count, median [IQR]	1445 [500–3731]	1007 (278–3731)	0.055
Imaging at baseline			
RVEF (%), mean±s.d.	42.5±11.1	43.80±10.40	0.096
LVEF (%), mean±s.d.	55.7±8.7	57.66±8.42	0.923
Treatment at baseline,			
Beta-blockers, n (%)	116 (57.5)	200 (37.9)	0.501
Anti-arrhythmic drugs, n (%)	102 (47.4)	82 (15.5)	**<0.001**
ICD, n (%)	78 (27.1)	218 (41.3)	**0.001**

Abbreviations as per Table 1

Overall, patients who underwent PVS were more likely to be male (55.9% vs 44.7%; p=0.002), probands (73.6% vs 49.8%; p<0.001), less likely to be variant carriers (58.0% vs 67.5%; p <0.001), more likely to have had a prior cardiac syncope (24.0% vs 9.1%; p<0.001), had more leads with TWI, and were less likely to have an ICD at baseline (27.1% vs 41.3%; p=0.001). Distribution of

the predicted risk of the study population according to the ARVC risk calculator has been reported in FigureS3.

PVS data

In 215 (88%) patients, a three extra stimuli PVS protocol was used, delivered at two RV sites (right ventricular apex and outflow tract) in 222 (89%). One hundred thirty-seven (47.6%) patients were inducible with a mean of 1.4 [1.2–1.5] sustained VT morphologies induced per patient. The median VT cycle length was 247 [220–280] ms, while the most common morphology was left bundle branch block (n=158, 72.5%), with a superior axis morphology (33.5%). **TableS1** summarizes procedural PVS data.

Inducible patients were younger (39.2±14.0 vs 42.7±14.7 years; p=0.037), disproportionately probands (81.0% vs 66.9%; p=0.007) and had more leads with TWI on ECG (4 [3–5] vs 2 [2–5]; p=0.003), more NSVT (56.2% vs 37.8%; p=0.002), a higher 24h PVC burden (1624 [600–4630] vs 1154 [475–2788]; p=0.026), and a lower RVEF (40.8±11.4 vs 43.9±10.6; p=0.027) than patients with no inducible VT. At multivariable analyses, however, the presence of NSVT at diagnosis was the only predictor for PVS inducibility (OR 2.095 [1.233–3.560]; p=0.006). The complete list of univariable and multivariable predictors of PVS inducibility is reported in **TableS2**.

Long-term outcomes

During a median follow-up of 5.31 [2.89–10.17] years, 120 (41.7%) patients experienced a sustained VA event **(Table 3)**. Patients who had a positive PVS were more likely to experience a sustained VA event than those in whom the PVS was negative (83 of 137, 60.6% versus 37 of 151, 24.5%; p<0.001). A total of 43 rapid VA/(aborted)SCD episodes were observed during follow-up, with no significant differences between those with and without a positive PVS (18.2% vs 11.9%, p=0.132). Overall, 23 (n = 19 sustained VT; n = 4 fast VT /(aborted)SCD) were experienced by patients without an ICD. At last follow-up, 196 (68.1%) had an ICD in place, 6 (2.1%) had

undergone heart transplant and 13 (4.5%) patients had died. **Figure1A** reports the cumulative freedom from first sustained VA in the whole cohort and **Figure1B** cumulative freedom from incident sustained VA stratified by PVS inducibility. **FigureS1** represents the timing of ICD implantation. **TableS3** reports ICD programming details. **FigureS4** reports cycle length concordance between the inducible VT at PVS and the observed clinical VT.

Table 3. Follow-up characteristics of the study cohort

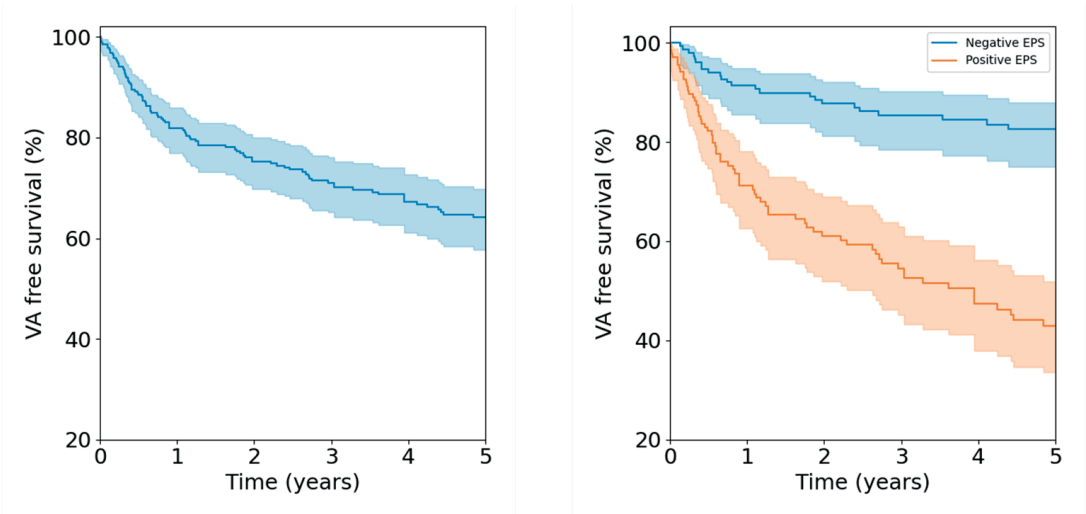
	Overall (n=288)	PVS + group (n=137)	PVS- group (n=151)	p
Follow-up time (years), median [IQR]	5.31 [2.89–10.17]	6.57 [2.73–10.45]	5.24 [3.21–9.59]	0.791
First sustained VA episode, n (%)	120 (41.7)	83 (60.6)	37 (24.5)	**<0.001**
Sustained VT, n (%)	26 (9.0)	18 (13.1)	8 (5.3)	**0.020**
ICD intervention, n (%)	89 (30.9)	62 (45.2)	27 (17.9)	**<0.001**
SCD, n (%)	5 (1.7)	3 (2.2)	2 (1.3)	0.575
Rapid VA/SCD episode, n (%)	43 (14.9)	25 (18.2)	18 (11.9)	0.132
VT ≥ 250 bpm, n (%)	13 (4.5)	7 (5.1)	6 (4.0)	0.643
ICD therapy, n (%)	23 (8.0)	13 (9.5)	10 (6.6)	0.370
SCD, n (%)	7 (2.4)	5 (3.6)	2 (1.3)	0.200
Cardiac transplant, n (%)	6 (2.1)	3 (2.2)	3 (2.0)	0.575
Death, n (%)	13 (4.5)	8 (5.8)	5 (3.3)	0.302

SCD: sudden cardiac death; VA: ventricular arrhythmia; VT: ventricular tachycardia; other abbreviations as per Table1

ARVC risk calculator and five-year outcomes

During the first 5-years of follow-up, 92 (34.0%) patients had a sustained VA event. Among the variables included in the previously published ARVC risk calculator, younger age (HR per year increase 0.98 [0.97–0.99], p=0.003), male sex (HR 1.78 [1.15–2.76]. p=0.009), presence of NSVT (2.09 [1.30–3.33], p=0.002), 24h PVC burden (HR per log increase 1.148 [1.03–1.29], p=0.016), and RVEF (HR per % increase 0.97 [0.95–0.99], p=0.001) were significantly associated with the development of sustained VAs in this time period. **FigureS2** reports the ARVC risk calculator calibration plot in this cohort, showing a strong correlation between the ARVC risk calculator predicted and observed arrhythmic risk in this cohort (r^2 of 0.94).

Figure 1.



Survival free from sustained VA. Cumulative survival free from sustained VA is presented with 95% confidence intervals (shaded area) in the overall population (panel A) and according to inducibility of sustained monomorphic VT on PVS (panel B) PVS: programmed ventricular stimulation; VA: ventricular arrhythmias

PVS and additional value in predicting five-year outcomes

Inducibility on PVS predicted sustained VA events over five years (HR 4.21 [2.64–6.71], p <0.001) on univariable Cox proportional hazards analyses. This predictive ability remained significant (HR 2.52 [1.58, 4.02], p < 0.001) after adjustment for the ARVC risk calculator predicted risk. The model combining ARVC risk calculator predicted risk and PVS inducibility (C-statistic 0.75) was superior to univariable Cox proportional hazard models using either PVS inducibility (C-statistic 0.66) or the ARVC risk calculator (C-statistic 0.72, LLR p < 0.001). Net reclassification improvement with a 5-year VA risk cut-off of <25% was 7% for the combined model relative to the ARVC risk calculator taken in isolation. The value of PVS for predicting 5-year sustained VA in the low/intermediate arrhythmic risk group (n=152; n=24 VAs in the 5-year follow-up) vs. high arrhythmic risk group (n=136; n=68 VAs in the 5-year follow-up) was as follows: low/intermediate risk group PPV 38.5% [25.4–51.6] and NPV 92.6% [87.4–97.5]; high risk group PPV 68.4% [58.5–78.3] and NPV 64.2% [51.2–77.2]. The sensitivity and specificity for PVS in the overall cohort were 75.7% [67.4-84.0] and 67.5% [60.75-74.3], respectively. The corresponding LRs were 2.3 for

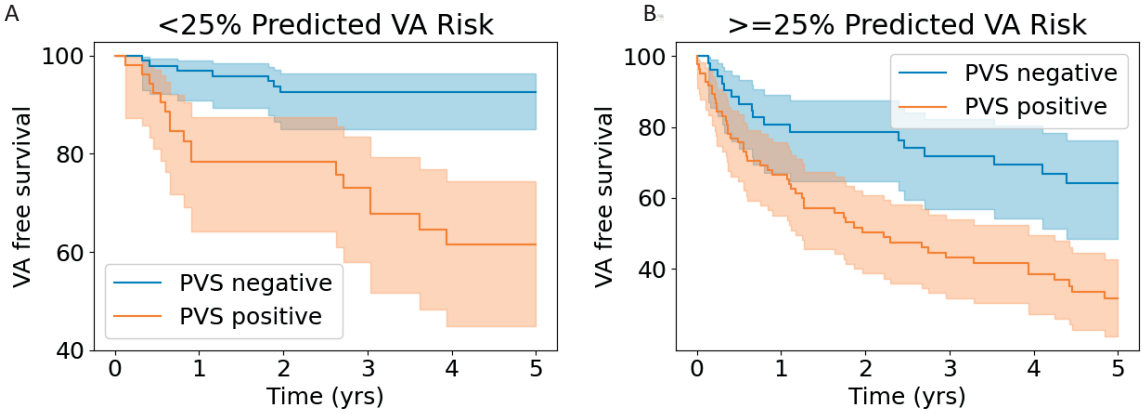
inducible (LR+) and 0.36 for non-inducible PVS (LR-). **Table4** illustrates post-PVS derived VA risk in patients with different pre-test predicted 5-year risk according to the ARVC risk calculator. For example, a patient with a 5-year ARVC risk prediction of 25% will have a 5-year post-test VA risk of 12.3% if non-inducible during PVS, and of 44.4% if inducible. Instructions for accessing a preliminary version of the planned online update for the current online calculator have been provided in the supplementary material. **Figure2 (Panel A&B)** show the cumulative survival free from sustained VA for inducible and non-inducible patients in the low/intermediate arrhythmic and high arrhythmic risk groups. A complete-case sensitivity analysis yielded similar results (Supplementary Materials Section B).

Table 4. Examples of Updated VA Risk according to PVS results (inducible or non-inducible) in patients with different a priori 5-year risks from the ARVC calculator (ARVCrisk.com).

Risk Bracket	Number of patients	Patients with sustained VA episodes*	Patients with rapid VA/SCD events**	5-year risk from ARVC calculator	PVS result	Updated VA Risk at 5-year
0–5.0	12 (4.2%)	1 (0.8%)	1 (2.2%)	5%	PVS + PVS –	10.8% 1.9%
5.1–10.0	31 (10.8%)	4 (3.3%)	3 (7.0%)	10%	PVS + PVS –	20.3% 3.8%
10.1–15.0	45 (15.6%)	11 (9.2%)	6 (14.0%)	15%	PVS + PVS –	28.9% 6.0%
15.1–20.0	41 (14.2%)	14 (11.7%)	2 (4.7%)	20%	PVS + PVS –	36.5% 8.3%
20.1–25.0	23 (8.0%)	10 (8.3%)	3 (7.0%)	25%	PVS + PVS –	43.4% 10.7%
25.1–30.0	30 (10.4%)	14 (11.7%)	8 (18.6%)	30%	PVS + PVS –	49.6% 13.4%
30.1–35.0	14 (4.9%)	6 (5.0%)	2 (4.7%)	35%	PVS + PVS –	55.3% 16.2%
35.1–40.0	12 (4.2%)	8 (6.7%)	3 (7.0%)	40%	PVS + PVS –	60.5% 19.4%
40.1–45.0	11 (3.8%)	7 (5.8%)	1 (2.2%)	45%	PVS + PVS –	65.2% 22.8%
45.1–50.0	13 (4.5%)	6 (5.0%)	2 (4.7%)	50%	PVS + PVS –	69.7% 26.5%
50.1–55.0	11 (3.8%)	7 (5.8%)	5 (11.6%)	55%	PVS + PVS –	73.8% 30.6%
55.1–60.0	7 (2.4%)	6 (5.0%)	1 (2.2%)	60%	PVS + PVS –	77.5% 35.1%

Abbreviations as per table 1 and 3. Sustained VA are defined as a composite of SCD, sustained VT (lasting ≥30 s or with hemodynamic compromise or requiring cardioversion), ventricular fibrillation/flutter (VF), or appropriate ICD intervention.
**% calculated on the total of sustained VA events (n=120)*
**% calculated on the total of rapid VA/SCD events (n=43)*

Figure 2



Survival free from VA in patient with: A)a calculated risk below 25%; B) a calculated risk of or above 25%; Cumulative survival free from sustained VA with 95% confidence intervals (shaded area) according to inducibility of sustained monomorphic VT on PVS in patients with a 5-year predicted ARVC risk below 25% (low/intermediate arrhythmic risk group) according to the online risk calculator (ARVCrisk.com). Abbreviations as per Figure 1

DISCUSSION

The main findings of this study can be summarized as follows: first, nearly half (47.6% percent) of this cohort of 288 patients referred for PVS had inducible sustained VA. Second, we found that inducibility at PVS could predict the occurrence of sustained VA over the 5 years follow-up. Finally, we showed that adding the inducibility on PVS to the current ARVC risk calculator significantly improved the model discrimination. Importantly, PVS was shown to have a high negative predictive value (92.6%) for incident sustained VA at 5 years in patients with an ARVC risk calculator predicted 5-year risk < 25%, and PVS results can be used together with the risk calculator to refine predictions in individual patients.

Risk Stratification in ARVC Patients

Once a diagnosis of ARVC is established, the next step in the clinical management is to assess the patient's risk of experiencing a sustained VA and to determine if an ICD is warranted.[3] In the past three decades multiple studies have aimed to identify the predictors of sustained VA in ARVC. A recent meta-analysis by Bosman et al. summarized those predictors and paved the way to the development of a novel risk stratification tool,[5,28] which integrates multiple non-invasive parameters.

This published ARVC risk calculator (ARVCrisk.com) aims to predict the 5-year risk of the first sustained VA event in definite ARVC patients. Multiple independent cohorts have reported good reliability of this risk calculator in different settings[6,7,9,10] and also confirmed its superiority to currently available risk stratification algorithms.[6,7] By including only patients referred for a PVS, this study cohort not surprisingly had a higher ARVC risk calculator predicted risk than the previous cohorts, in which the ARVC risk calculator was developed and validated. Nonetheless, the ARVC risk calculator showed good performance (calibration slope=0.92 and C-statistic 0.72) in predicting the 5-year outcomes in this subpopulation as well.

PVS in ARVC arrhythmic risk stratification

The utility of sustained VT inducibility on PVS as a predictor of VA in ARVC has drawn significant attention in prior literature. While some investigators have reported its clinical utility in predicting long-term arrhythmic outcomes,[6,14,15,18] other studies found a PPV as low as 35% for PVS in this patient population.[20] Due to its invasive nature, precluding its use in all patients, PVS was not included in the original ARVC risk calculator. The possibility that integrating the results of PVS with the ARVC risk calculator might further improve risk estimates was postulated soon after its publication.[13]

The primary purpose of this study was to better define the contemporary role of PVS in risk stratification of patients with ARVC who do not present with a sustained VA. This study is unique

not only because of its large size (with an international cohort of 288 primary prevention ARVC patients it is, to our knowledge, the largest report of PVS in ARVC to date), but also because we examined the incremental predictive value of PVS on the recently published ARVC risk calculator. A strong correlation between sustained VT inducibility and arrhythmic outcomes was clearly observed during a median follow-up of more than 5 years. Patients in whom sustained arrhythmias were induced during PVS had a 4-fold risk of sustained VA events during follow-up. Furthermore, we showed that PVS results provide additional value when integrated with the existing ARVC risk calculator. A model combining both PVS and the ARVC risk calculator predicted risk was in fact superior at predicting 5-year arrhythmic outcomes than either of these two predictors alone.

Clinical Implications

An important clinical question that may arise is the role of PVS in guiding primary prevention ICD placement in ARVC patients. The results of this study suggest that PVS may be of value in the risk stratification process of patients who have a low/intermediate predicted risk (<25% at 5-years) based on the ARVC risk calculator (Table4). In patients with VA predicted risks at the extremes (either very high or very low per the calculator), an invasive PVS procedure would likely be of limited use. Conversely, this additional stratification tool can be of greatest use in clinical decision-making process in patients with an intermediate predicted risk. In this study, a negative PVS had a high NPV (92.6%) in patients at low/intermediate predicted risk (less than 25% at 5 years) per the risk calculator. This makes a robust argument in favor of the use of a negative PVS to support a clinical decision not to implant an ICD in patients in whom the risk score suggests a low or an intermediate predicted risk. Additionally, PVS results can be directly integrated to the ARVC risk calculator in an adjusted approach to refine risk prediction in individual patients using the Bayes' theorem. This personalized approach can help in selecting patients who are most likely to benefit from this invasive procedure to facilitate the therapeutic decision regarding ICD use.

Limitations:

All centers involved are tertiary, high volume referral centers and some degree of selection bias in patient enrollment cannot be ruled out. Furthermore, patients were selected for this study based on their referral for PVS, which relied on clinical decision-making of individual cardiologists. Consequently, patients at very low predicted risk, less advanced disease stages and family members are under-represented in this cohort referred for an invasive risk stratification method. The generalizability of our findings to these other types of ARVC patients is unclear. As with any clinical predictive model, validation in an external cohort will be important for the clinical implementation of this additive method to the ARVC risk calculator. Additionally, the predicted outcome of any sustained VA, which included sustained VA and ICD-treated arrhythmia, cannot be considered a strict surrogate of SCD. Specifically for rapid VA/(aborted) SCD, rates were numerically larger for patients with PVS+ than for patients with PVS- but the difference did not reach statistical significance, expressing limited power or a lack of predictive ability. Adequately powered studies aimed at addressing this specific outcome would be of great use in the future. However, due to the appropriate use of ICDs and more timely diagnosis, SCD has fortunately become a rare event in ARVC patients. The primary aim of most studies has therefore shifted away from overall/cardiovascular mortality and SCD towards the overall burden of sustained VA events, for which a clear difference between patients with positive and negative PVS is observed.

CONCLUSION:

In this multicenter cohort of primary prevention patients with ARVC referred for PVS, sustained VT inducibility on PVS significantly improved the prediction of arrhythmic outcomes 5 years after diagnosis beyond the ARVC risk calculator. A two-step approach integrating PVS to the risk calculator's prediction can further refine risk estimates improving the decision-making process regarding ICD implantation in selected patients with ARVC.

Funding:

The Johns Hopkins ARVD/C Program is supported by the Leonie-Wild Foundation, the Leyla Erkan Family Fund for ARVD Research, the Hugh Calkins, Marvin H. Weiner, and Jacqueline J. Bernstein Cardiac Arrhythmia Center the Dr. Francis P. Chiramonte Private Foundation, the Dr. Satish, Rupal, and Robin Shah ARVD Fund at Johns Hopkins, the Bogle Foundation, the Healing Hearts Foundation, the Campanella family, the Patrick J. Harrison Family, the Peter French Memorial Foundation, the Wilmerding Endowments, Fondation Leducq and UL1TR001079 (NCATS).This work was performed during Dr. Gasperetti's tenure as the Wilton W. Webster Fellowship in Clinical Cardiac Catheter Ablation Fellow of the Heart Rhythm Society; Dr. Cadrin-Tourigny's work is supported by the Philippa and Marvin Carsley cardiology research chair and the Montreal Heart Institute Foundation. The Zurich ARVC Program is supported by the Georg und Bertha Schwyzer-Winiker Foundation, Baugarten Foundation, Leonie-Wild Foundation, Swiss Heart Foundation and Swiss National Science Foundation (SNF); Prof. Platonov's work in the Nordic ARVC Registry is supported by The Swedish Heart Lung Foundation (grant #20200674) and support from the Swedish state under the ALF-agreement. The Dutch ARVC registry (Profs Van Tintelen and Wilde and Dr te Riele) are funded by the Netherlands Cardiovascular Research Initiative, with support of the Dutch Heart Foundation (grant CVON2015-12/2018-30 eDETECT/PREDICT2).

Acknowledgments:

We thank the ARVC patients and families who have made this work possible. We also thank Drs Jeroen van der Heijden MD, PhD; Peter Loh MD, PhD; Mimount Bourfiss MD, Rob W. Roudijk MD and Ms. Machteld J. Boonstra Msc.

Conflict-of-interest disclosures:

C Tondo serves as member of the Advisory Board of Medtronic, Boston Scientific. He receives lecture and proctoring fees from Medtronic, Abbott, Boston Scientific.

AMS received educational grants through his institution from Abbott, Bayer Healthcare, Biosense Webster, Biotronik, Boston Scientific, BMS/Pfizer, and Medtronic; and speaker /advisory board /consulting fees from Abbott, Bayer Healthcare, Daiichi-Sankyo, Medtronic, Novartis and Pfizer.

C Tichnell is a consultant for StrideBio Inc. CAJ is a consultant for Pfizer, Inc, Tenaya Inc., and StrideBio, Inc. HC is a consultant for Medtronic Inc., Biosense Webster, Pfizer, StrideBio, and Abbott. He receives research support from Boston Scientific Corp. C Tichnell and CAJ receive salary support from this grant. JCT is a consultant for Tenaya Inc.

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Supplementary Material

Supplementary Tables

Table S1

Electrophysiological study data	288 (100)
Number of stimulation sites (n=249) Two sites, n (%)	222 (89.1%)
Number of extra-stimuli (n), median [IQR] Up to 2 Up to 3 Up to 4+	 16 (6.5%) 215 (88.1%) 13 (5.3%)
Shortest coupling (ms), median [IQR]	210 [200–230]
Isoproterenol use, n (%)	97 (33.7)
High dose isoproterenol, n (%)	9 (3.1)
PVS+ with isoproterenol, n (%)*	38 (39.2)
Pts experiencing events, n (%)	22/38
PVS- with isoproterenol, n (%)*	59 (60.8)
Pts experiencing events, n (%)	8/59
Inducible sustained monomorphic VT, n (%)	137 (47.6)
Overall number of VTs**, n	218
LBBB, n (%)	158 (72.5)
Inferior Axis, n (%)	48 (22.0)
Superior Axis, n (%)	73 (33.5)
Unknown, n (%)	37 (17.0)
RBBB, n (%)	13 (6.0)
Polymorphic, n (%)	18 (8.3)
Cycle length available, n (%)	137 (62.8)
Median cycle length (ms), median [IQR]	248 [220 – 280]
Cycle length ≥300 ms	7/137 (5.1)
Cycle length 240 - 299 ms, n (%)	79/137 (57.6)
Cycle length 200-239 ms, n (%)	38/137 (27.7)
Cycle length <200 ms, n (%)	13/137 (9.5)
AH interval, median [IQR]	81 [65–100]
HV interval, median [IQR]	45 [40–52]
Contextual VT Ablation, n (%)	26 (9.0)

***Percentage calculated on the total of patients undergoing isoproterenol PVS**

**Details of VT morphology were not available in 29 cases

AH: atrial-to-His; HV: His-to-ventricle; LBBB: left bundle branch block; PVS: programmed ventricular stimulation; RBBB: right bundle branch block; VT: ventricular tachycardia;

Table S2

Predictors of sustained VT inducibility at PVS

	OR [C.I.]	p	aOR [C.I.]	p
Age (per year increase)	0.98 [0.97–1.00]	**0.038**	0.98 [0.97–1.00]	0.073
Male sex	1.36 [0.85–2.17]	0.199		
Caucasian ethnicity	0.68 [0.28–1.62]	0.383		
Proband status	2.11 [1.23–3.65]	**0.007**	1.44 [0.76–2.72]	0.265
Desmosomal variant carrier	1.16 [0.70–1.93]	0.566		
Recent cardiac syncope	1.49 [0.86–2.56]	0.154		
Total number of TWI (per unit increase)	1.19 [1.05–1.34]	**0.005**	1.13 [0.98–1.31]	0.091
NSVT at diagnosis	2.12 [1.32–3.39]	**0.002**	2.10 [1.23–3.56]	**0.006**
24-h PVC count* (per log unit increase)	1.01 [0.96–1.25]	0.166		
RVEF (%) (per % increase)	0.97 [0.95–0.99]	**0.029**	0.98 [0.96–1.01]	0.123
LVEF (%) (per % increase)	1.00 [0.97–1.02]	0.801		
ARVC Risk Score (%) (per % increase)	0.65 [0.41–0.91]	**<0.001**		

aOR: adjusted odds ratio; LVEF: left ventricular ejection fraction; NSVT: non sustained ventricular tachycardia; OR: odds ratio; PVC: premature ventricular contraction; PVS: programmed ventricular stimulation; RVEF: right ventricular ejection fraction; TWI: T wave inversion; VT: ventricular tachycardia.

*logarithmic relationship

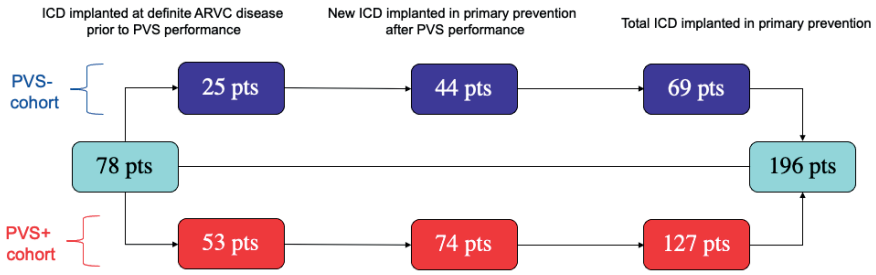
Table S3

ICD programming available (n = 148 pts)				
	Overall Cohort (n = 148)	EPS+ (n = 88)	EPS- (n = 60)	p
Monitor zone (ms), median [IQR]	350 [330–375]	333 [324–400]	351 [333–375]	0.419
VT Therapy zone (ms), median [IQR]	307 [293–329]	300 [292–330]	310 [300–322]	0.986

Supplementary figures

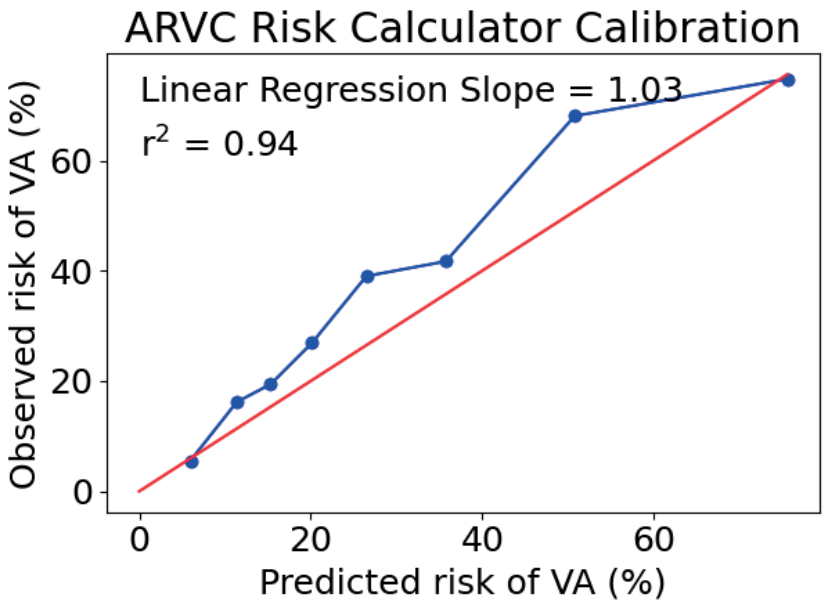
Figure S1

ICD implantation flowchart



ICD: implantable cardioverter defibrillator, ARVC: arrhythmogenic right ventricular cardiomyopathy, PVS: programmed ventricular stimulation

Figure S2-Calibration plot illustrating the performance of the ARVC risk score for 5-year sustained VA prediction.



VA: ventricular arrhythmia

Figure S3

Distribution of predicted risks according to the ARVC risk calculator

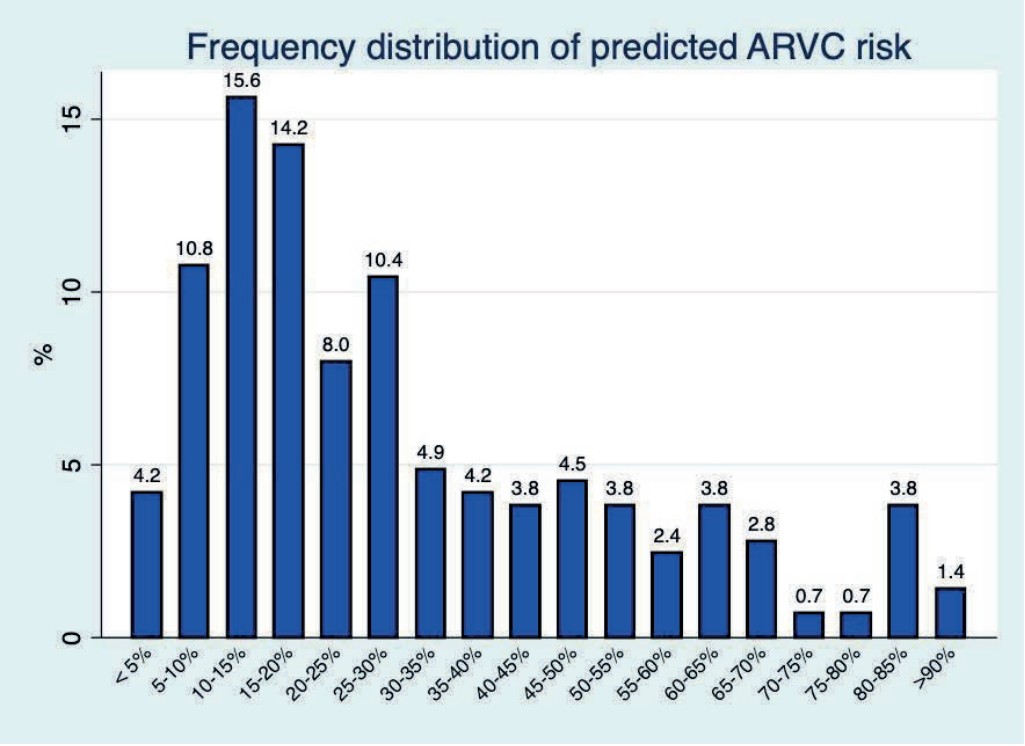
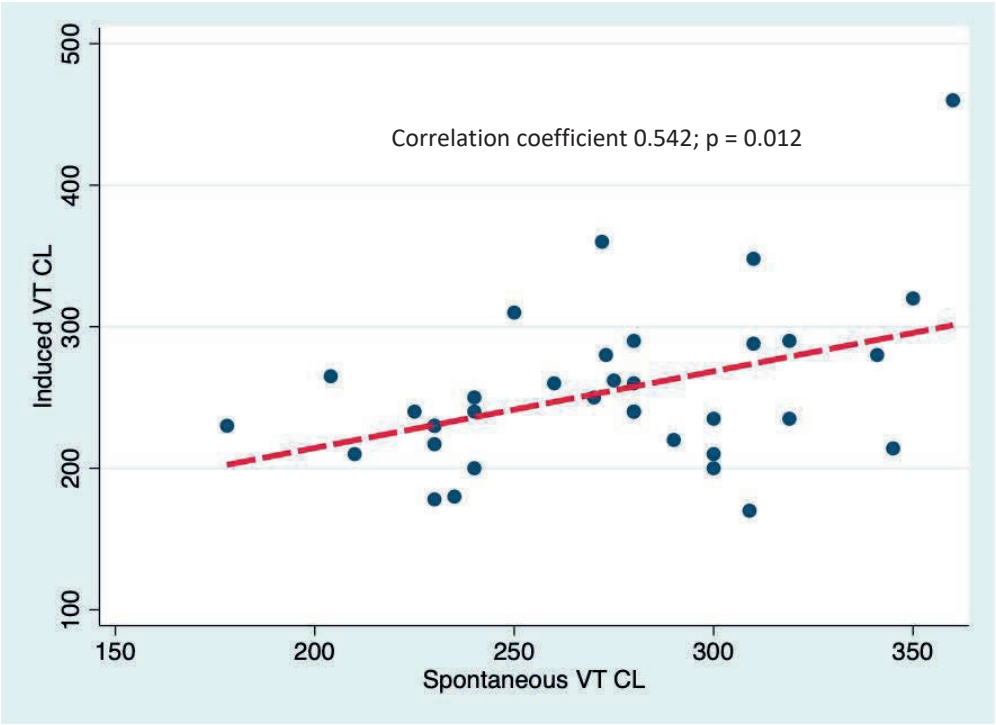


Figure S4

Correlation between cycle length of PVS induced VT and spontaneous observed VT in the study cohort



Section B – Complete Case Analyses

Population: n=227 pts; n=98 pts with PVS+

- Total cohort:
  - Sensitivity 76.6 [66.6 – 85.5];
  - Specificity 70.3 [63.2 – 77.5];
  - Positive predictive value 54.5 [44.6 – 64.3];
  - Negative predictive value 85.8 [79.8 – 91.8];
  - Positive likelihood ratio 2.58;
  - Negative likelihood ratio 0.33.
- A priori risk <25%:
  - Positive predictive value 45.8 [31.4 – 60.2];
  - Negative predictive value 92.9 [87.6 – 98.3];
  - Positive likelihood ratio 3.35;
  - Negative likelihood ratio 0.25.
- A priori risk >=25%:
  - Positive predictive value 62.4 [49.3 – 75.6];
  - Negative predictive value 70.6 [56.5 – 84.7];
  - Positive likelihood ratio 1.79;
  - Negative likelihood ratio 0.44.

- Association between ARVC calculator / PVS and outcomes
  - Concordance for ARVC calculator alone: 0.688
  - Concordance for PVS alone: 0.674
    - HR of PVS alone: 4.71 [2.69 – 8.25]; $p < 0.001$
  - Concordance for ARVC + PVS: 0.738
    - HR of PVS: 2.70 [1.54 – 4.73]; $p < 0.001$
  - LLR test for superiority of combined model: Test Statistic 7.369; $p = 0.007$

No significant differences in the overall results were observed between the complete-case and the complete cohort using multiple imputation by chained equation analyses.

Section C –Patient characteristics by registry

Johns Hopkins Registry (n=108)	
Age, mean±s.d.	35.2±12.8
Male sex, n (%)	50 (46.3)
Caucasian, n (%)	106 (98.1)
Proband Status, n (%)	74 (68.5)
Recent cardiac syncope, n (%)	26 (24.1)
Leads with TWI on ECG	
TWI in ≥3 precordial leads, n (%)	73 (67.6)
TWI in ≥2 inferior leads, n (%)	25 (23.1)
NSVT at diagnosis, n (%)	57 (52.8)
24-h PVC count, median [IQR]	1647 [357–5160]
Imaging at baseline	
RVEF (%), mean±s.d.	40.6±11.6
LVEF (%), mean±s.d.	58.0±7.3
Beta-blockers at baseline, n (%)	44 (40.7)
Events at follow up, n (%)	52 (48.1)

Italian Registry (n=87)	
Age, mean±s.d.	47.8±13.3
Male sex, n (%)	66 (75.9)
Caucasian, n (%)	87 (100)
Proband Status, n (%)	76 (87.4)
Recent cardiac syncope, n (%)	25 (28.7)
Leads with TWI on ECG	
TWI in ≥3 precordial leads, n (%)	54 (62.1)
TWI in ≥2 inferior leads, n (%)	25 (28.7)
NSVT at diagnosis, n (%)	26 (29.9)
24-h PVC count, median [IQR]	1100 [500–2341]
Imaging at baseline	
RVEF (%), mean±s.d.	46.2±8.9
LVEF (%), mean±s.d.	52.2±10.1
Beta-blockers, n (%)	43 (49.4)
Events at follow up, n (%)	36 (41.4)

Montreal site from the Canadian HIRO registry (n=8)	
Age, mean±s.d.	43.3±14.4
Male sex, n (%)	4 (50.0)
Caucasian, n (%)	2 (25.0)
Proband Status, n (%)	6 (75.0)
Recent cardiac syncope, n (%)	6 (75.0)
Leads with TWI on ECG	
TWI in ≥3 precordial leads, n (%)	5 (62.5)
TWI in ≥2 inferior leads, n (%)	2 (25.0)
NSVT at diagnosis, n (%)	5 (62.5)
24-h PVC count, median [IQR]	1623 [1445–3343]
Imaging at baseline	
RVEF (%), mean±s.d.	35.0±17.7
LVEF (%), mean±s.d.	52.6±8.0
Beta-blockers, n (%)	2 (25.0)
Events at follow up, n (%)	6 (75.0)

Dutch Registry (n=43)	
Age, mean±s.d.	42.7±13.9
Male sex, n (%)	20 (46.5)
Caucasian, n (%)	42 (97.7)
Proband Status, n (%)	22 (51.2)
Recent cardiac syncope, n (%)	13 (30.2)
Leads with TWI on ECG	
TWI in ≥3 precordial leads, n (%)	27 (62.8)
TWI in ≥2 inferior leads, n (%)	14 (32.6)
NSVT at diagnosis, n (%)	26 (60.5)
24-h PVC count, median [IQR]	2057 [975–4008]
Imaging at baseline	
RVEF (%), mean±s.d.	43.3±10.2
LVEF (%), mean±s.d.	56.6±7.5
Beta-blockers, n (%)	14 (32.6)
Events at follow up, n (%)	15 (34.9)

Swiss Registry (n=27)	
Age, mean±s.d.	39.7±14.5
Male sex, n (%)	14 (51.9)
Caucasian, n (%)	27 (100)
Proband Status, n (%)	23 (85.2)
Recent cardiac syncope, n (%)	8 (29.6)
Leads with TWI on ECG	
TWI in ≥3 precordial leads, n (%)	21 (87.5)
TWI in ≥2 inferior leads, n (%)	2 (7.4)
NSVT at diagnosis, n (%)	14 (51.9)
24-h PVC count, median [IQR]	1005 [500–3386]
Imaging at baseline	
RVEF (%), mean±s.d.	37.7±14.5
LVEF (%), mean±s.d.	57.2±10.0
Beta-blockers, n (%)	13 (48.1)
Events at follow up, n (%)	7 (25.9)

Nordic Registry (Norway and Sweden) (n=15)	
Age, mean±s.d.	41.8±18.8
Male sex, n (%)	7 (46.7)
Caucasian, n (%)	14 (93.3)
Proband Status, n (%)	11 (73.3)
Recent cardiac syncope, n (%)	7 (46.7)
Leads with TWI on ECG	
TWI in ≥3 precordial leads, n (%)	9 (60.0)
TWI in ≥2 inferior leads, n (%)	3 (20.0)
NSVT at diagnosis, n (%)	7 (46.7)
24-h PVC count, median [IQR]	1735 [109–10000]
Imaging at baseline	
RVEF (%), mean±s.d.	40.3±13.9
LVEF (%), mean±s.d.	53.9±10.2
Beta-blockers, n (%)	7 (46.7)
Events at follow up, n (%)	4 (26.7)

CHAPTER 7

ASSOCIATION OF PREMATURE VENTRICULAR CONTRACTION BURDEN ON SERIAL HOLTER MONITORING AND ARRHYTHMIC RISK IN PATIENTS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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JAMA Cardiol. *2022 Apr 1;7(4):378-385. doi: 10.1001/jamacardio.2021.6016 PMID: 35195686*

ABSTRACT

Importance: A high burden of premature ventricular contractions (PVCs) at disease diagnosis has been associated with an overall higher risk of ventricular arrhythmias (VAs) in arrhythmogenic right ventricular cardiomyopathy (ARVC). Data regarding dynamic modification of PVC burden at Holter follow-up and its impact on arrhythmic risk in ARVC is scarce.

Objective: To describe changes in the PVC burden and to assess whether serial Holter monitoring is dynamically associated with sustained VAs during follow-up in ARVC patients.

Design, Settings, and Participants: In this cohort stuy, patients with a definite ARVC diagnosis, available Holter at disease diagnosis, and at least two additional Holters during follow-up were enrolled from six ARVC registries in North America and Europe.

Main outcome and measure: the association between pre-specified variables retrieved at each Holter-follow up (overall PVC burden, presence of sudden PVC increases [spikes, defined as: absolute increase in PVC burden ≥5000/24-hours or a relative ≥75% increase, with an absolute increase ≥1000 PVCs], presence of non-sustained ventricular tachycardia (NSVT), use of beta-blockers and class III AADs) and sustained VAs occurring within 12 months from that Holter was assessed using a mixed logistical model.

Results: In 169 enrolled ARVC patients (36.3±15.0 y.o., 56.2% male), a total of 723 Holter exams (4 [4–5] Holters/patient) were performed over a follow-up of 54 [42–63] months, detecting 75 PVC spikes and 67 sustained VAs. The PVC burden decreased significantly from the first to second Holter (mean -2906 PVC/24h; $p<0.001$) and remained stable thereafter (overall $p=0.876$). A model including 24-h PVC burden (OR 1.494 [1.102–2.028], $p=0.010$), PVC spikes (OR 6.283 [2.764–14.283]; $p<0.001$) and NSVT (OR 2.215 [1.092–4.491]; $p=0.027$) at each follow-up Holter showed a high association with sustained VA occurrence in the following 12 months.

Conclusions and relevance: In patients with ARVC, changes in parameters derived from each Holter performed during follow-up can dynamically predict the risk of sustained VAs in the 12 upcoming months.

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited cardiac disease characterized by ventricular arrhythmias (VAs), an increased risk of sudden cardiac death, and progressive fibro-fatty replacement of the myocardium[1,2].

The first step in disease management after ARVC diagnosis is risk stratification to determine if a primary prevention implantable cardioverter defibrillator (ICD) placement is warranted. [3–5]. Recently, a novel risk stratification tool was developed (www.arvcrisk.com) [6,7]. This calculator allows clinicians to determine the 5-year arrhythmic risk of patients without previous sustained VAs at the time of diagnosis. Several external cohorts have subsequently confirmed the predictive accuracy of this risk calculator [6,8–11].

Premature ventricular contractions (PVCs) are a hallmark of ARVC. A high PVC burden has also been associated with an increased risk of sustained VAs and ICD interventions in patients with ARVC [12]. Therefore, the assessment of the PVC burden at the time of disease diagnosis is recommended in current clinical risk stratification strategies [3–5]. Additionally, PVC count on a Holter monitor represents one of the components of the recently developed ARVC risk calculator [6,7]. However, ARVC is a progressive disease and the weight of risk markers can vary during follow-up [13]. Whether individual variations in PVC burden may predict arrhythmic events has not been previously investigated. Thus, the purpose of this study was twofold: to describe changes in PVC burden over time in ARVC patients following disease diagnosis, and to determine whether a changing PVC burden on follow-up Holter monitors can be used to predict subsequent arrhythmic events.

METHODS:

Study design

The ARVC registries of 6 high-volume referral academic institutions (Johns Hopkins University, Baltimore; Montreal Heart Institute, Montreal; Ospedale Universitario Careggi, Florence; Centro Cardiologico Monzino, Milan; Ospedali Riuniti, Ancona; Azienda Sanitaria Universitaria Giuliano Isontina, Trieste) from 3 different countries (United States of America, Canada, Italy) were screened for all consecutive patients fulfilling the following inclusion criteria:

1. Definite ARVC diagnosis in accordance to the 2010 International Task Force Criteria (ITFC)[14];
2. Availability of a 24-h Holter monitor at disease diagnosis, defined as "baseline Holter";
3. Availability of at least two additional Holters over the 5 years following disease diagnosis, with a maximum 18-month interval between any two Holters.

Ethical, review board approval and patient consent were obtained at each center, in accordance to local regulations. The study was performed in accordance with the Declaration of Helsinki. Data supporting these findings is available upon reasonable request to the corresponding author.

Data Collection

For each patient fulfilling inclusion criteria, demographic (age, gender, proband status), clinical (history of any VA or cardiac syncope preceding disease diagnosis), and ARVC diagnostic features (ITFC criteria fulfillment; RVEF% at disease diagnosis retrieved as per previous methods from this group[6]) were extracted. For patients with available genetic testing, pathogenic/likely pathogenic variants in one of the genes associated with ARVC were reported after adjudication according to the American College of Medical Genetics and Genomics guidelines[15].

From every available Holter monitor, the 24-h PVC burden and the presence of non-sustained ventricular tachycardia (NSVT) and/or sustained VA was collected.

The use of class I-C/class III anti-arrhythmic drugs (AADs) and of beta-blockers was assessed at baseline and at every Holter follow-up. Sustained VA events occurring during follow-up were recorded. All time dependent variables were collected with the time of disease diagnosis set as time zero reference. Patient follow-up started at the time of disease diagnosis and ended 12 months after the last available Holter.

Definitions and study endpoints.

The primary aims of this study were:

1. To describe the variation of the PVC burden over time in a population of definite ARVC patients
2. To assess the dynamic association of Holter derived parameters with the occurrence of a sustained VA event in the 12 months immediately following each Holter.

We also repeated these analyses with patients stratified according to their history of sustained ventricular arrhythmia at the time of ARVC diagnosis ("primary prevention" versus "secondary prevention") as a secondary aim.

Sustained VA was defined as a ventricular tachycardia (VT) lasting ≥30 s or with hemodynamic compromise requiring cardioversion, ventricular fibrillation/flutter (VF), or an appropriate ICD intervention. A PVC spike was defined as a) an absolute increase in PVC burden ≥5000 PVCs and/or b) a relative % increase ≥75% from the preceding Holter, with an absolute increase of at least 1000 PVCs. The presence of a PVC spike was assessed for every Holter available at follow-up, upon comparison with the PVC burden of the Holter immediately preceding. Sensitivity analysis using 50% and 100% relative burden increase for defining PVC spikes are included in **Section 1-A** of the supplemental materials.

Statistical Analysis:

All analysis were performed using STATA v 14.0 (STATA Corp, Lakeway Drive, Texas, USA). Categorical variables were reported as count (percentage). Distribution of continuous variables was tested using a Shapiro-Wilk test. Continuous variables were reported as mean±standard deviation (sd) or as median [inter-quartile range (IQR)], in accordance with variable distribution. Comparisons between numerical variables were performed using a paired T test or Wilcoxon signed-rank test. Overall progression over time of the PVC burden was tested through linear regression.

Associations between pre-determined clinical and Holter-derived variables of interest (namely: male sex; overall PVC burden; presence of a PVC spike; presence of NSVT; use of beta-blockers; use of class III AADs) and the occurrence of a sustained VA event in the subsequent 12 months were tested using mixed effect logistic regression. Variables of interest were treated as fixed effects, while patient identity was treated as random effect to control for inter-patient variability. Only variables reaching significance of $p < 0.05$ in the single-variable models were considered for inclusion in a subsequent multiple-variable model. Variables included in the multi-variate, mixed-effect logistic regression model were chosen using stepwise, backward-selection of predictors and minimization of Akaike's information criteria (AIC) (with a difference of AIC of 2 used as a threshold for continued addition of variables). The discriminative performance of the final model was measured using C-Statistic (area under the curve of the receiver operator curve). Agreement between predicted and observed outcomes was evaluated graphically using calibration plots. The predictive capability of the final model was then graphically expressed through predictive curves derived from the fixed margins of the final multivariate mixed-effect logistic model using STATA's "-margin-" function.

RESULTS

Overall patient cohort

A total of 169 patients were enrolled in the study. **Table1** summarizes the baseline characteristics of the study cohort. The mean age was 36.3±15.0 years and 56.2% of the patients were male. At baseline, 50.9% of the cohort was on beta-blockers and 23.1% on class IC/III AADs. Among the enrolled patients, a total of 723 Holters were performed (median number of Holters/patient 4 [4–5]). The median inter-Holter time was 12 [11–15] months, while the follow-up time of the study was 54 [42–63] months. **Table2** summarizes Holter specifics of the study cohort.

Table 1

Baseline Characteristics (n=169)	
Age (years), mean±s.d.	36.3±15.0
Male sex, n (%)	95 (56.2)
Proband Status, n (%)	128 (75.7)
2010 TFC fulfillment	
Class I–Morphology	
Major, n (%)	*78 (47.3)*
Minor, n (%)	*35 (21.2)*
Class II–Tissue characterization	
Major, n (%)	*27 (16.4)*
Minor, n (%)	*5 (3.0)*
Class III–Repolarization abnormalities	
Major, n (%)	*89 (52.7)*
Minor, n (%)	*50 (29.6)*
Class IV–Depolarization abnormalities	
Major, n (%)	*9 (5.5)*
Minor, n (%)	*46 (27.9)*
Class V–Arrhythmias	
Major, n (%)	*31 (18.8)*
Minor, n (%)	*101 (61.2)*
Class VI–Family history	
Major, n (%)	*96 (56.8)*
Minor, n (%)	*13 (7.7)*
Pathogenic/Likely Pathogenic Mutation, n (%)	85 (50.3)
PKP2, n (%)	*54 (32.0)*
DSP, n (%)	*19 (11.2)*
DSG2, n (%)	*8 (4.7)*
DES, n (%)	*2 (1.2)*
FLNC, n (%)	*2 (1.2)*
Recent cardiac syncope, n (%)	24 (14.2)
TWI, median [IQR]	3 [2–4]
NSVT at diagnosis, n (%)	61 (36.7)
24-h PVC count, median [IQR]	5852 [4409–7295]
History of sustained VT at diagnosis, n (%)	47 (27.8)
RVEF at CMR (%), mean±s.d.	46.0±12.2
Treatment at baseline,	86 (50.9)
Pts on beta-blockers, n (%)	39 (23.1)
Pts on AADs, n (%)	*36 (21.3)*
	6 (3.6)

Sotalolo, n (%)	*3 (1.8)*
Class Ic, n (%)	73 (43.2)
Amiodarone, n (%)	
ICD, n (%)	

AADs: anti-arrhythmic drugs; CMR: cardiac magnetic resonance; DES: Desmin; DSG2: Desmoglein-2; DSP: Desmoplakin; FLNC: Filamin-C; ICD: implantable cardioverter defibrillator; NSVT: non-sustained ventricular tachycardia; PKP2: Plakophyllin-2; PVC: premature ventricular complex; RVEF: right ventricular ejection fraction; TFC: Task Force Criteria; TWI: T wave invesion; VT: ventricular tachycardia

PVC burden variation and PVC Spikes

Shown in **Figure1** is the median PVC count per 24 hours on the baseline and on subsequent Holters obtained during follow-up. The study cohort presented with a high PVC burden at disease diagnosis (mean burden/24h 5852 PVC/24h, 95% C.I. [4409–7295]). A significant reduction in the 24h-PVC burden was observed at the first follow-up Holter performed at a median of 6 [6–12] months from disease diagnosis (mean reduction -2906 PVC/24h, 95% C.I. [1581–4231]; p<0.001). Following this initial drop, the 24-h PVC burden remained stable during subsequent Holter evaluations (overall p=0.876; **Figure1**). No differences in PVC burden reduction between patients on and off beta-blocker therapy were observed (**FigureS1)**.

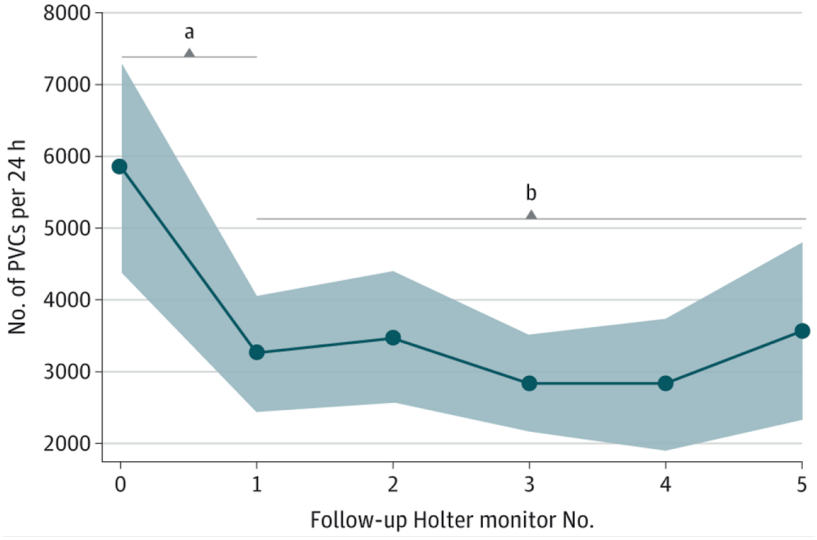
Table 2

Holter Data	
Number of Holters/patient, median	4 [4–5]
3 Holters, n (%)	42 (24.9)
4 Holters, n (%)	59 (34.9)
5 Holters, n (%)	47 (27.8)
6 Holters, n (%)	21 (12.4)
Median inter-Holter time, months	12 [11–15]
Patients experiencing PVC Spikes, n (%)	69 (39.6)
Holter distribution	
Baseline Holter, n (%)	*169 (100)*
PVC burden, mean (95% C.I.)	5852 [4409–7295]
NSVT, n (%)*	61 (36.7)
B-blockers, n (%)*	86 (50.9)
Class Ic/III, n (%)*	39 (23.1)
F.U. Holter 1 (< 12 m.o), n (%)	*146 (86.4)*
PVC burden, mean (95% C.I.)	3248 [2439 – 4057]
PVC spike, n (%)*	10 (6.8)
NSVT, n (%)*	40 (27.4)
B-blockers, n (%)*	79 (54.1)
Class Ic/III, n (%)*	35 (24.0)
F.U. Holter 2 (12 – 24 m.o.), n (%)	*149 (88.2)*
PVC burden, mean (95% C.I.)	3477 [2561 – 4393]
PVC spike, n (%)*	27 (18.1)
NSVT, n (%)*	48 (32.2)
B-blockers, n (%)*	81 (54.4)

Class Ic/III , n (%)*	37 (24.8)
F.U. Holter 3 (24 – 36 m.o.), n (%)	*122 (72.2)*
PVC burden, mean (95% C.I.)	2838 [2165 – 3510]
PVC spike, n (%)*	13 (10.7)
NSVT, n (%)*	33 (27.0)
B-blockers, n (%)*	59 (48.4)
Class Ic/III, n (%)*	30 (24.6)
F.U. Holter 4 (36 – 48 m.o.), n (%)	*86 (50.9)*
PVC burden, mean (95% C.I.)	2824 [1904 – 3745]
PVC spike, n (%)*	14 (16.3)
NSVT, n (%)*	18 (20.9)
B-blockers, n (%)*	43 (50.0)
Class Ic/III, n (%)*	22 (25.6)
F.U. Holter 5 (48 – 60 m.o.), n (%)	*51 (30.2)*
PVC burden, mean (95% C.I.)	3564 [2332 – 4795]
PVC spike, n (%)*	11 (21.6)
NSVT, n (%)*	13 (25.5)
B-blockers, n (%)*	26 (51.0)
Class Ic/III , n (%)*	11 (21.6)

*m.o.: months; NSVT: non-sustained ventricular tachycardia; PVC: premature ventricular complex; *percentage calculated on the amount of Holters available at that follow up point*

Figure1



Mean and 95% C.I. of the 24h PVC burden assessed at Holter during follow-up. After an initial drop (a: p<0.001), the PVC burden remained stable over time (b: p=0.88). Holter Follow Up Grouping Time: 0) At disease diagnosis (n=169); 1) Within first 12 m.o. from diagnosis (n=146); 2) 13-24 m.o. from diagnosis (n=149); 3) 24-36 m.o. from diagnosis (n=122); 4) 36-48 m.o. from diagnosis (n=86); 5) 48-60 m.o. from diagnosis (n=50). PVC: premature ventricular contraction

A total of 75 PVC spikes were identified in 67 (39.6%) of the 169 patients enrolled in this study (n=32 fulfilling both definition -a- and -b- for a spike; n=43 fulfilling definition -b- for a spike). In Holters in which a PVC spike was observed, the median increase in PVC per 24-h burden was +4900 [2400–7139] PVC/24h, i.e. a median increase of 319% [142–1279]. Baseline

characteristics of patients with and without PVC spikes at follow-up were comparable, with the exception of PVC burden at baseline, which was higher in the former (3851 [1241–9979] vs 1553 [366–7000] PVC/24h; p=0.011) (**Table S1**).

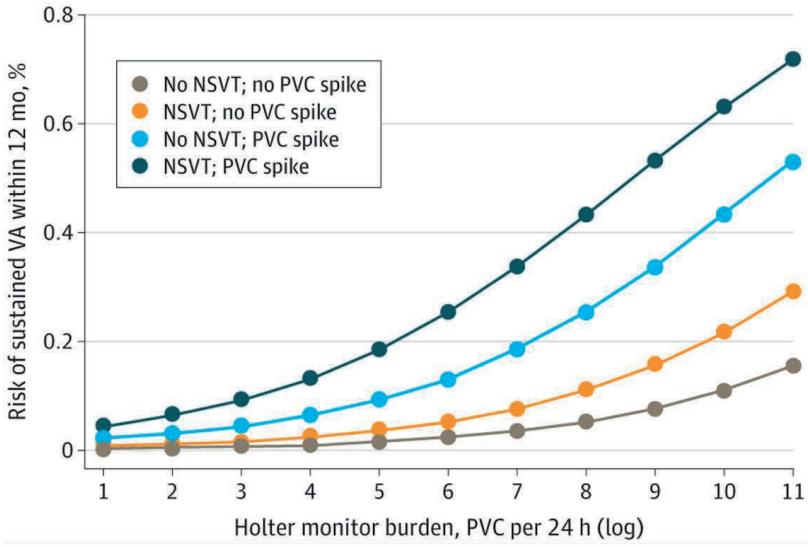
Arrhythmic Events association with Holter Results.

A total of 67 sustained VA events in 57 (33.7%) different patients were observed during follow-up (n=14 sustained VT; n=50 appropriate ICD interventions; n=3 VF). The vast majority (94.1%) occurred within 12 months of the previous Holter test, with only 4 events occurring outside the 12 month window (all between disease diagnosis and first follow-up Holter). **Table S2** reports the characteristics of patients with and without VA events during follow-up. Twenty-two additional ICDs were implanted during the study follow-up.

Tables S3-S8 reports the association of each tested parameter individually with the occurrence of a sustained VA event in the upcoming 12 months. Occurrence of a sustained VA event in the 12 months immediately following each Holter was associated with greater 24-h PVC burden (OR per log increase 2.2 [1.6–2.9]; p<0.001), the presence of PVC spikes (OR 13.1 [6.0–28.3]; p<0.001) or episodes of NSVT (OR 4.1 [2.3–7.2]; p<0.001). The combination of these three parameters (PVC count, PVC spike, and NSVT) derived from any Holter at follow-up demonstrated a good association with sustained VA events in the subsequent 12 months (C Statistic: 0.891 [0.853–0.929). **Table S8** reports the final model.

As shown in **Figure2**, the risk of a sustained VA event within 12 months of a Holter increased with the complexity of arrhythmias observed on that Holter. Notably, however, risk was greater in the presence of PVC spikes (6-fold increase) than NSVT (only 2-fold). For example, a patient with a PVC burden of 3000 PVC/24h (log≈8), NSVT and a PVC spike would have > 40% risk of a sustained VA event within 12 months, while a patient with the same PVC burden but without NSVT or the presence of a PVC spike would have only around a 5% risk.

Figure2



Probability of a sustained VA event within 12 months from an 24-h ECG Holter performed at follow-up depending on three variables: a) 24-h PVC burden (on x axis, reported as a logarithmic (log_e) value); b) presence/absence of NSVT; c) presence/absence of a PVC spike. The presence of a PVC spike has the strongest association with a sustained VA event in the upcoming 12 months and results more important than the 24-h PVC burden for any 24-h PVC burden.

Of note, the inclusion of the 24-h PVC burden at disease diagnosis in the model did not improve its performance. The final model performance in the population stratified by whether or not the patient had experienced a sustained VA at disease diagnosis is reported in **Table S10-S11**. **.** Model performance in patients with and without ICDs, regardless of their arrhythmic status at baseline have been reported in **Table S12-13**. **Figure S2** reports the calibration plot for the final model.

DISCUSSION:

This is the first study to extensively address the changes in the 24h PVC burden during follow-up in a multicenter cohort of patients with ARVC and to assess the dynamic association of the parameters derived from follow-up Holter exams with the occurrence of sustained VA events in the subsequent 12 months.

The main results of this study may be summarized as follows. First, a significant drop in the overall 24h PVC burden within the first 12 months of follow-up from disease diagnosis was

observed in the majority of ARVC patients. Second, although the overall PVC burden remained generally stable over the remaining follow-up, the occurrence of sudden, self-limiting increases in PVCs (PVC spikes) at individual Holters was present in approximately one third of patients. At each Holter monitor, both absolute 24h PVC burden and presence of PVC spike or NSVT was strongly associated with the occurrence of sustained VAs in the subsequent 12 months. Together, these results suggest serial Holter monitoring is an effective and accessible strategy that should be considered for dynamic arrhythmia risk assessment and management in ARVC patients.

Holter monitor findings over time

This study describes for the first time the modifications in PVC burden observed in patients with ARVC, over a long follow-up comprising in median of four Holter reassessments per patient, performed approximately every 12 months. In our cohort, the PVC burden dropped significantly at the first re-assessment after disease diagnosis and remained stable thereafter. This initial PVC burden reduction may be attributed to a combination of endurance and high level endurance sports restriction and/or initiation of pharmacological therapy. At the time of disease diagnosis and after first Holter assessment, in fact, 50.9% of patients were started on beta-blockers and 23.1% on class Ic/III AADs, with those percentages remaining stable at all times of follow-up. A similar drop in PVC burden has been previously observed in a small cohort of high-end athletes with ARVC undergoing physical de-training [10]. In that setting, the drop in PVC burden was greater in patients started on beta-blockers and/or AADs, but even in patients off medications a significant reduction was observed. Our findings confirm and extend these findings to a broader population of ARVC patients. Unfortunately, given the multicentered nature of this study, the dose of physical exercise during follow-up was not routinely quantified in a standardized way. Therefore, the relative weights and the competing benefits of pharmacological treatment and exercise restriction on PVC burden reduction could not be quantified. Further dedicated prospective studies will be required to specifically answer this question.

Almost 40% of patients experienced sudden, self-limiting, increases in their PVC burden (PVC spike) on one or more follow-up Holters. ARVC is now considered a progressive disease with a relapsing-remitting evolution, with phases of inflammation and increased arrhythmic activity (the so called "hot phases") [16–20]. To this day, hot phases have been tracked through the assessment of atypical symptoms at patient admission (i.e. myocarditis like episodes), troponin elevations, cardiac imaging exams, and histology assessment [18,20–22], of which PVC spikes might represent useful Holter red flags, with potential impact on management.

Holter PVC count association with arrhythmic risk

Following a definite diagnosis of ARVC, physicians face the complex task of estimating the arrhythmic risk of each individual patient. If this is deemed to be low, discontinuation of physical exercise and initiation of beta blocker therapy may be considered an adequate treatment. Conversely, a higher arrhythmic risk may warrant the managing physician discussing the option of implanting an ICD with the patient.

Recently, a novel arrhythmic risk stratification tool for ARVC patients has been developed[6,7]. This tool allows prediction of the 5-year arrhythmic risk of individual patients using clinical and instrumental data, retrieved at the time of disease diagnosis. Still, given the progressive nature of the disease, the profile of patients with ARVC may worsen over time, requiring dynamic risk assessment in primary prevention. In particular, patients deemed at low arrhythmic risk at baseline, and thus not implanted with an ICD, may benefit from a dynamic reassessment during follow-up, in order to facilitate timely capture sudden changes in arrhythmic propensity and reconsider the indication for a device. Likewise dynamic risk prediction could help in the management of patients already implanted with ICDs, aiding in the titration of beta-blockers and anti-arrhythmic medications to minimize the incidence of appropriate shocks.

The magnitude of the PVC burden at disease diagnosis has been directly associated with the risk of sustained VAs and ICD interventions in patients with ARVC and it is part of the recently

developed risk calculator [6,12]. In this study, we postulated that the arrhythmic data from each Holter performed during follow-up, and mainly the observed PVC burden, may be dynamically associated with changes in the arrhythmic risk profile of individual patients. In our cohort, we observed that the overall magnitude of the PVC burden and the presence of PVC spikes and/or of NSVT at each follow-up Holter were reliably associated with the occurrence of sustained VAs in the upcoming 12 months, with sudden PVC spikes representing the most important red flag. The presence of PVC spikes was associated with upcoming VA events across all subgroups, regardless of ICD status at and previous history of sustained VA events at baseline.

The findings of this study provide additional strong support for the systematic use of PVC burden reassessment through serial Holters, to dynamically re-evaluate the arrhythmic risk of patients with ARVC during follow-up. PVC burden changes may be in fact used in the clinical practice to integrate the original risk stratification performed at disease diagnosis to assess the need for further changes in life-style, for pharmacologic management, and for an ICD at any point during follow-up. A clinical management strategy integrating a baseline assessment at the time of disease diagnosis using the ARVC risk score calculator and periodic re-evaluation can therefore be envisioned.

For patients without a history of sustained VAs ("primary prevention" ARVC patients), a risk assessment can be performed at disease diagnosis using the ARVC risk calculator. Depending on the predicted risk and through a shared decision-making algorithm accounting for individual values and preferences of the patient, the placement of an ICD in primary prevention may be considered. A yearly re-assessment of the arrhythmic risk through Holters may then be used to monitor the progression and changes in the arrhythmic profile. This approach may be of particular value when placement of an ICD is not performed at presentation. The results of annual Holter monitoring can then be used to guide further therapeutic interventions such as more aggressive exercise restriction, initiation or an increase in the dose of beta blocker therapy, initiation of antiarrhythmic therapy, or reconsideration of the value of ICD implantation.

Future directions and next perspectives

While this dynamic approach seems intuitive and of clinical value, it must be recognized that additional studies are needed to completely assess the impact of Holter guided management on arrhythmic outcomes. A prospective trial, with the per-protocol yearly performance of an Holter for dynamic arrhythmic risk reassessment and a decision making algorithm based on Holter findings, represents the next step that needs to be performed. A similar study, including a quantitative exercise exposure assessment and structural (i.e. through cardiac ultrasounds / MRI) and/or serum biomarker (i.e. troponin leaks, natriuretic peptides) assessments in the presence of a PVC spike would provide additional insights on those points that the current study was not designed to address.

Furthermore, a prospective collection of Holter data in a suitable format for machine learning analysis and artificial intelligence processing would allow the potential recognition of more exact PVC cut-offs and of even additional variables associated with an increased arrhythmic risk during dynamic follow up that the naked human eye may have missed.

Finally, it should also be noted that several brands of ICD implement algorithms to quantify the PVC burden of implanted patients. All PVC burdens presented in our study were derived from Holters and data regarding consistency between Holter-derived and ICD-estimated PVC burdens in current literature are scarce. Assessing this correlation in future studies will be critical, because it would allow to further translate a dynamic, PVC-burden based arrhythmic risk re-assessment into the every-day clinical setting. If a good correlation between Holter- and ICD-estimated PVC burden were to be present, it would in fact be reasonable to use continuous ICD-estimation of the PVC burden (potentially accessible even from remote, during telemedicine visits) to track the changes in the arrhythmic risk of patients with ARVC implanted with an ICD, similarly to what is currently done with the estimation of fibrillation burden by implantable loop recorder in the setting of many atrial fibrillation clinics.

Limitations:

The main aim of the study was to assess the effectiveness of using Holters as a dynamic metric of the arrhythmic risk profile of patients with ARVC. The retrospective nature of this study represents a first limitation that should be acknowledged. Although serial monitoring is routinely used at the involved institutions for all patients with ARVC and patients from the entire spectrum of arrhythmic risk in ARVC were included in the study, a certain degree of bias in patients selection could not be completely ruled out. These findings should therefore be interpreted primarily as hypothesis generating. Finally, due to the retrospective nature of the study, physical exercise modifications over follow-up were not assessed in a standard fashion and therefore the relative weight of their impact on arrhythmic outcomes and the potential competing benefit with the use of beta-blockers and AADs could not be assessed. Further prospective trials building up on these findings are therefore needed.

CONCLUSION

In a multicentered cohort of patients with ARVC undergoing serial Holter evaluation, a significant drop in the overall 24h PVC burden was observed within 12 months from disease diagnosis. Occurrence of individual self-limiting PVC spikes was observed in more than one third of patients and NSVT was observed on follow-up Holters in 20% of patients. The absolute 24h PVC burden and the presence of a PVC spike and NSVT at each Holter performed during follow-up were strongly associated with the occurrence of VAs in the immediately following 12 months. A strategy employing yearly Holter follow-up to dynamically assess the individual patient arrhythmic risk profile at follow-up may be an effective integration to the current risk stratification tools for arrhythmic risk in ARVC.

Funding:

The authors wish to acknowledge funding from Fondation Leducq and UL1TR001079 (NCATS). The Johns Hopkins ARVD/C Program is supported by the Leonie-Wild Foundation, the Leyla Erkan Family Fund for ARVD Research, the Dr. Francis P. Chiramonte Private Foundation, the Dr. Satish, Rupal, and Robin Shah ARVD Fund at Johns Hopkins, the Bogle Foundation, the Healing Hearts Foundation, the Campanella family, the Patrick J. Harrison Family, the Peter French Memorial Foundation, and the Wilmerding Endowments. This work was performed during Dr. Gasperetti's tenure as the Wilton W. Webster Fellowship in Clinical Cardiac Catheter Ablation Fellow of the Heart Rhythm Society; Dr. Cadrin-Tourigny's work is supported by the Philippa and Marvin Carsley research chair.

Acknowledgments:

Dr. Gasperetti had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. We are grateful to the ARVC patients who have made this work possible.

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Table S1

Characteristics at Disease Diagnosis			
	ARVC patients with PVC spikes (n=67)	ARVC patients without PVC spikes (n=102)	p
Age (years), mean±s.d.	37.5±14.9	35.5±15.0	0.381
Male sex, n (%)	37 (55.2)	58 (56.9)	0.834
Proband Status, n (%)	54 (80.6)	74 (72.6)	0.233
Pathogenic/Likely Pathogenic Variant, n (%)	33 (49.2)	52 (51.0)	0.826
Recent cardiac syncope, n (%)	13 (19.4)	11 (10.8)	0.116
TWI, median [IQR]	3 [2–4]	3 [2–4]	0.755
nsVT at diagnosis, n (%)	29 (43.9)	32 (32.0)	0.118
24-h PVC count, median [IQR]	3851 [1241–9979]	1553 [366–7000]	**0.011**
History of SVT at diagnosis, n (%)	19 (28.4)	28 (27.5)	0.898
RVEF at CMR (%), mean±s.d.	44.9±12.7	46.8±11.8	0.338

Table S2

Characteristics at Disease Diagnosis			
	ARVC patients with VA events (n=57)	ARVC patients without VA event (n=112)	p
Age (years), mean±s.d.	34.0±14.4	37.4±15.1	0.166
Male sex, n (%)	35 (51.4)	60 (53.6)	0.332
Proband Status, n (%)	49 (85.9)	79 (70.5)	**0.027**
Recent cardiac syncope, n (%)	11 (9.8)	13 (22.8)	**0.022**
TWI, median [IQR]	4 [3 – 5]	3 [2 – 4]	**0.002**
nsVT at diagnosis, n (%)	27 (48.2)	34 (30.9)	**0.029**
24-h PVC count, median [IQR]	5000 [2240 – 8000]	1437 [333 – 6047]	**<0.001**
History of SVT at diagnosis, n (%)	20 (35.1)	27 (24.1)	0.132
RVEF at CMR (%), mean±s.d.	43.7±10.2	47.2±12.9	0.102
LVEF at CMR (%), mean±s.d.	53.4±8.1	55.1±8.2	0.247

Table S3

Per Holter Event Predictor			
Fixed Effects			
	OR	C.I.	p
PVC at Holter (log)	2.189	[1.636–2.929]	**<0.001**
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.760	0.343	[0.313–1.842]

Table S4

Per Holter Event Predictor			
Fixed Effects			
	OR	C.I.	p
PVC Spike	13.071	[6.036–28.307]	**<0.001**
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.890	0.350	[0.413–1.919]

Table S5

Per Holter Event Predictor			
	Fixed Effects		
	OR	C.I.	p
NSVT	4.110	[2.333–7.240]	**<0.001**
	Random Effects		
	Estimate	Standard Error	C.I.
Patient	0.441	0.420	[0.068–2.852]

Table S6

Per Holter Event Predictor			
	Fixed Effects		
	OR	C.I.	p
Use of BB-blockers	1.010	[0.574–1.773]	0.973
	Random Effects		
	Estimate	Standard Error	C.I.
Patient	0.570	0.358	[0.166–1.952]

Table S7

Per Holter Event Predictor			
	Fixed Effects		
	OR	C.I.	p
Use of ClassIII AADs	1.191	[0.630–2.253]	0.590
	Random Effects		
	Estimate	Standard Error	C.I.
Patient	0.554	0.365	[0.153–2.012]

Table S8

Per Holter Event Predictor			
	Fixed Effects		
	OR	C.I.	p
Male sex	1.042	[0.585–1.856]	0.888
	Random Effects		
	Estimate	Standard Error	C.I.
Patient	0.574	0.357	[0.169–1.942]

Table S9

Holter predictors of an SVA event in the upcoming 12 months			
	Fixed Effects		
	OR	C.I.	p
24-h PVC burden (log)	1.498	[1.104–2.034]	**0.010**
Presence of a PVC spike	6.196	[2.743–13.993]	**<0.001**
Presence of NSVT	2.289	[1.100–4.514]	**0.026**
	Random Effects		
	Estimate	Standard Error	C.I.
Patient	0.882	0.347	[0.408–1.907]

Table S10

Final model in primary prevention patients with ARVC (n=122)

Holter predictors of an SVA event in the upcoming 12 months			
Fixed Effects			
	OR	C.I.	p
24-h PVC burden (log)	1.388	[0.955–2.017]	0.086
Presence of a PVC Spike	8.276	[2.663–25.715]	**<0.001**
Presence of nsVT	2.297	[0.907–5.818]	0.080
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.893	0.499	[0.299–2.669]

Table S11
Final model in secondary prevention patients with ARVC (n=47)

Holter predictors of an SVA event in the upcoming 12 months			
Fixed Effects			
	OR	C.I.	p
24-h PVC burden (log)	1.673	[0.986–2.839]	*0.056*
Presence of a PVC Spike	4.150	[1.159–14.863]	**0.029**
Presence of nsVT	2.171	[0.712–6.621]	0.173
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.884	0.507	[0.287–2.718]

Table S12
Final model performance in patients with ARVC with no ICD at baseline (n=96)

Holter predictors of an SVA event in the upcoming 12 months			
Fixed Effects			
	OR	C.I.	p
24-h PVC burden (log)	1.504	[0.954–2.375]	*0.079*
Presence of a PVC Spike	7.835	[2.127–28.858]	**0.002**
Presence of nsVT	2.191	[0.692–6.940]	0.182
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.689	0.436	[0.081–5.834]

Table S13

Final model performance in patients with ARVC implanted with ICD at baseline (n=73)

Holter predictors of an SVA event in the upcoming 12 months			
Fixed Effects			
	OR	C.I.	p
24-h PVC burden (log)	1.600	[1.033–2.479]	**0.035**
Presence of a PVC Spike	4.472	[1.527–13.099]	**<0.001**
Presence of nsVT	2.056	[0.835–5.059]	0.117
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.806	0.436	[0.279–2.326]

Section 1-A

The following tables reports the final model performance if the following definition of a PVC spike is used:

- a) an absolute increase in PVC burden ≥5000 PVCs and/or
- b) a relative % increase ≥50% from the preceding Holter, with an absolute increase of at least 1000 PVCs.

Holter predictors of an SVA event in the upcoming 12 months			
Fixed Effects			
	OR	C.I.	p
24-h PVC burden (log)	1.532	[1.128–2.082]	**0.006**
Presence of a PVC Spike	5.343	[2.387–11.962]	**<0.001**
Presence of nsVT	2.223	[1.103–4.479]	**0.025**
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.889	0.343	[0.418–1.893]

C-statistic: 0.889 [0.850–0.927]

The following tables reports the final model performance if the following definition of a PVC spike is used:

- a) an absolute increase in PVC burden ≥5000 PVCs and/or
- b) a relative % increase ≥100% from the preceding Holter, with an absolute increase of at least 1000 PVCs.

Holter predictors of an SVA event in the upcoming 12 months			
Fixed Effects			
	OR	C.I.	p
24-h PVC burden (log)	1.557	[1.151–2.106]	**0.004**
Presence of a PVC Spike	5.189	[2.352–11.447]	**<0.001**
Presence of nsVT	2.213	[1.108–4.422]	**0.024**
Random Effects			
	Estimate	Standard Error	C.I.
Patient	0.837	0.342	[0.376–1.864]

Harrel's C: 0.880 [0.840–0.920]

Figure S1

Figure S1 reports PVC burden modification during follow up stratifying patients by beta-blocker therapy. No significant difference in the trend of reduction of the PVC burden was observed between patients on and off beta-blocker therapy.

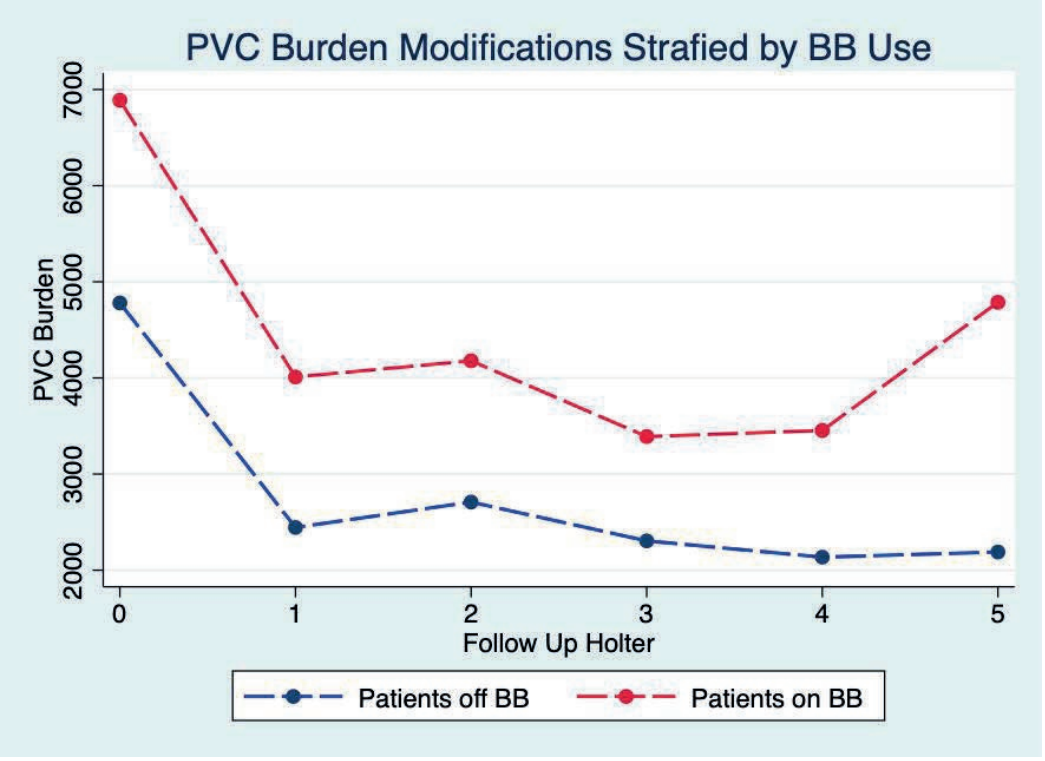
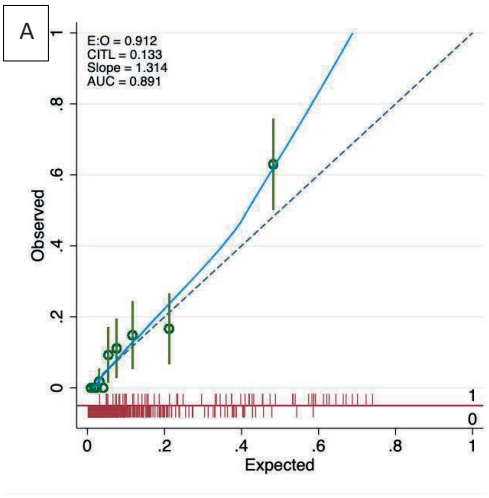
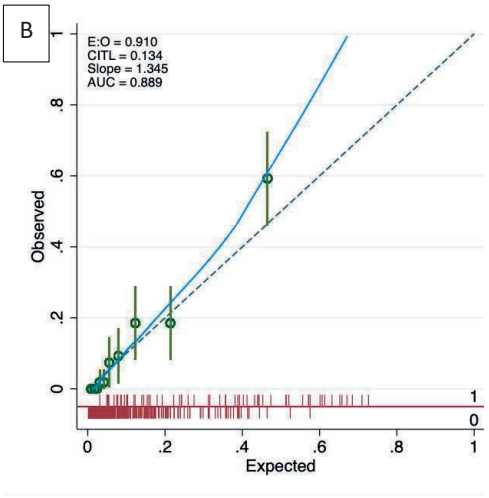
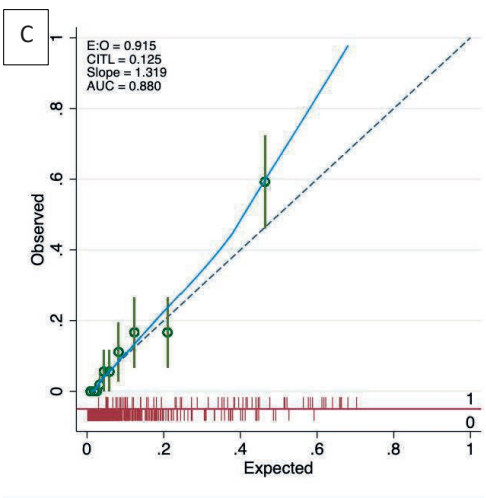


Figure S2 (Panel A / B / C).
Calibration plots for:

- a) Final model, using the PVC spike definition from the main manuscript;
- b) Final model, using a PVC spike definition with 50% increase as % increase threshold;
- c) Final model, using a PVC spike definition with 100% increase as % increase threshold;







The overall results of the 3 model are comparable, both in overall significance and in with the model included in the manuscript using a 75% increase as a percentage cut-off presenting a slightly superior discrimination.

CHAPTER 8

LONGITUDINAL PREDICTION OF VENTRICULAR ARRHYTHMIC RISK IN PATIENTS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

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Circ Arrhythm Electrophysiol. *2022 Nov; 15;(11):e011207. doi: 10.1161/CIRCEP.122.011207 PMID: 3615818*

ABSTRACT:

Background: The arrhythmogenic right ventricular cardiomyopathy (ARVC) risk calculator stratifies risk for incident sustained ventricular arrhythmias (VA) at the time of ARVC diagnosis. However, included risk factors change over time, and how well the ARVC risk calculator performs at follow-up is unknown.

Methods: This was a retrospective analysis of patients with definite ARVC and without prior sustained VA. Risk factors for VA including age, non-sustained ventricular tachycardia, premature ventricular complex burden, T-wave inversions on electrocardiogram, cardiac syncope, right ventricular function, therapeutic medication use, and exercise intensity were assessed at the time of 2010 Task Force Criteria based ARVC diagnosis and upon repeat evaluations. Changes in these risk factors were analyzed over 5-year follow-up. The 5-year risk of VA was predicted longitudinally using 1) the baseline ARVC risk calculator prediction, 2) the ARVC risk prediction calculated using updated risk factors, and 3) time-varying Cox regression. Discrimination and calibration were assessed in comparison to observed VA event rates.

Results: 408 ARVC patients experiencing 132 primary VA events were included. Matched comparison of risk factors at baseline versus at 5-years of follow-up revealed decreased burdens of premature ventricular complexes (-1,200/day) and non-sustained ventricular tachycardia (-14%). Presence of significant right ventricular dysfunction and number of T-wave inversions on electrocardiogram were unchanged. Observed risk for VA decreased by 13% by 5-years follow-up. The baseline ARVC risk calculator's ability to predict 5-year VA risk worsened during follow-up (c-statistic, 0.83 at diagnosis versus 0.68 at 5-years). Both the updated ARVC risk calculator (c-statistics of 0.77) and time-varying Cox regression model (c-statistic 0.77) had strong discrimination. The updated ARVC risk calculator overestimated 5-year VA risk by an average of +6%.

Conclusion: Risk factors for VA in ARVC are dynamic, and overall risk for incident sustained VA decreases during follow-up. Up-to-date risk factor assessment improves VA risk stratification.

INTRODUCTION:

Arrhythmogenic right ventricular cardiomyopathy (ARVC), the most common form of arrhythmogenic cardiomyopathy, is a heterogeneous genetic disease characterized by fibro-fatty infiltration of the myocardium and the development of potentially lethal ventricular arrhythmias (VA)[1]. While ARVC is rare with a prevalence of only 1 in 1,000 to 1 in 5,000[2, 3], it accounts for 10% to 20% of sudden cardiac deaths (SCD) in young adults[4]. The judicious implantation of cardioverter defibrillators (ICDs) in high-risk patients with ARVC is thus a core component of disease management. However, device-related risks are well known and may be particularly impactful among ARVC patients who are generally diagnosed at younger ages[5, 6]. Prospectively identifying those patients who are at high risk for VA, and consequently more likely to derive benefit from ICD placement, is therefore of critical importance in implantation decision making. The ARVC risk calculator was recently proposed as a tool for individualized VA risk assessment[7]. The Cox proportional hazards-based ARVC risk calculator incorporates a series of seven clinical predictors (age, sex, right ventricular ejection fraction (RVEF), premature ventricular complex (PVC) burden on ambulatory cardiac monitoring, history of non-sustained ventricular tachycardia (NSVT), the total number of T-wave inversions (TWI) in precordial and inferior leads on electrocardiogram (ECG), and history of recent cardiac syncope) to determine a particular patient's likelihood of developing incident VA over the five year period following his or her ARVC diagnosis. This tool has demonstrated excellent ability to discriminate between low- and high-risk ARVC patients (c-statistic, of 0.77) and has been increasingly adopted into clinical ICD decision making algorithms[8]. However, ARVC is a progressive condition and clinical predictors included in the ARVC risk calculator may be dynamic[9-11]. How reliably this tool performs at subsequent evaluations after initial diagnosis is therefore unknown, limiting longitudinal risk assessment in these patients.

To address this question, we analyzed data from a large, multi-center cohort of ARVC patients without prior sustained VA which included repeat clinical, imaging, and electrophysiologic

assessments during routine follow-up. We hypothesized that VA risk predictors change over time, and that incorporation of these changes is necessary for accurate longitudinal risk prediction in ARVC.

METHODS:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design

We conducted an observational, retrospective, longitudinal cohort study in accordance with the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) statement[12].

Study Population

The study population comprised patients enrolled in the Johns Hopkins ARVC registry and the Netherlands Arrhythmogenic Cardiomyopathy (ACM) registry. In brief, consecutive patients from were included in the current study if (i) they were diagnosed with definite ARVC by the 2010 Task Force Criteria[1], (ii) had not experienced prior sustained VA at the time of ARVC diagnosis, and (iii) had longitudinal clinical follow-up of at least 1 day. This study conforms to the Helsinki declaration and was approved by local ethics and/or institutional review boards. Participants signed informed consent to have their data included in the registry.

Data Collection

As described previously[7], data were collected independently by each participating center using uniform definitions (**Supplemental Table I**). Outcomes and baseline characteristics were adjudicated at each center via review of clinical visit documentation, ECG tracings, ICD interrogation tracings, ambulatory cardiac monitoring reports, echocardiography reports, cardiac magnetic resonance imaging (CMR) reports, as well as medical and death records. Genetic variants were adjudicated according to the American College of Medical Genetics and Genomics guidelines by consensus of specialists in cardiac genetics[13]. Additional longitudinal data from subsequent

clinical follow-up were also collected, including repeat ECG tracings, echocardiography reports, ambulatory cardiac monitoring reports, prescribed medication reviews, and exercise histories. Due to high prevalence of ICD implantation in these patients and the resulting low number of repeat CMR studies performed during follow-up, longitudinal CMR data was not included.

Study Outcomes

Consistent with the published ARVC risk calculator[7], the primary outcome was first sustained VA following confirmed ARVC diagnosis. Sustained VA was defined as a composite of SCD, sudden cardiac arrest, spontaneous sustained ventricular tachycardia [VT; lasting ≥30 seconds (s) at ≥100 beats per minute (bpm) or with hemodynamic compromise requiring cardioversion], ventricular fibrillation, or appropriate ICD intervention (defined as anti-tachycardia pacing or defibrillation). Incident heart transplantation, cardiovascular mortality, and all-cause mortality were also collected.

Predictor Variables and the ARVC Risk Calculator

Variables included in the ARVC risk calculator were considered[7]. These include sex, age, recent cardiac syncope (defined as transient loss of consciousness and postural tone with spontaneous recovery with a likely arrhythmic mechanism within the preceding 6-months), presence of NSVT (defined as hemodynamically stable VT at ≥100 bpm, for ≥3 beats <30 s), burden of PVCs on most recent 24-hour ambulatory cardiac monitoring, extent (defined as sum) of TWI on anterior and inferior leads on ECG, and RVEF. Each predictor variable was determined at the time of diagnosis, defined as within one year of ARVC diagnosis but always before arrhythmic outcome, and at each follow-up visit. The timing of clinical follow-up was based upon the discretion of local physicians. Due to the limited availability of CMR-derived RVEF assessments during follow-up, the ARVC risk calculator was modified by replacing the RVEF variable with presence of moderate or severe RV dysfunction as a dichotomous, echocardiographically-derived variable (**Supplemental Methods I**). We will refer to this model as the "modified ARVC risk calculator", the formula of which is presented in **Equation 1**,

$$P(\text{Sustained VA by 5 years}) = 1 - 0.791^{\exp(\text{Prognostic Index})} \quad (1)$$

where prognostic index is calculated according to **Equation 2**.

$$(2) \quad \text{Prognostic Index} = -\text{Age} * 0.022 + \text{Male sex} * 0.558 + \text{Presence of NSVT} * 0.754 + \text{Cardiac Syncope} * 0.441 + \text{Burden TWI} * 0.096 + \ln(24\ \text{PVC count}) * 0.278 + \text{Presence of Moderate or Severe RV dysfunction} * 0.351 - 2.176$$

Discrimination of this model was assessed using concordance-based c-statistic and 5-fold cross validation.

Longitudinal VA Risk. Risk Predictor Trends, and Risk Prediction at Follow-up

Longitudinal trends in risk factors included in the ARVC risk calculator, as well as those of alternative risk modifiers (left ventricular ejection fraction (LVEF), prescription rates of anti-arrhythmic medications and beta blockers, and level of athletic activity) were assessed by plotting the change in risk factor values relative to their patient-matched value at the time of diagnosis as a function of follow-up time. A window size equal to 2 years was used for the moving average, and analysis was limited to those patients for whom relevant testing/evaluation was available at both time of diagnosis and at follow-up. Patient data was not censored by VA event.

Longitudinal VA risk was estimated by repeating Kaplan-Meier analysis at each follow-up time out to 5-years. Patients were included in these analyses if they remained free of VA at the assessed follow-up time. Longitudinal prediction of 5-year VA risk was performed using three methods for interval follow-up risk estimation:

1) Baseline ARVC risk calculator: risk prediction calculated using only risk factors available at the time of diagnosis.
2) Updated ARVC risk calculator: risk prediction calculated using the most recent set of risk factors available at the time of follow-up evaluation.
3) Time-varying Cox regression: non-proportional Cox regression model that predicts risk as a function of both changing risk factors and the baseline hazard function (**Supplemental Methods II**).

Statistical analysis

Analyses were performed using PyCharm software version 2021.2 (JetBrains Inc., Boston, MA, USA) and open-source Pandas data analysis library, Lifelines survival analysis library, and statsmodels statistical modeling library. Missingness in data for the predictors included in the baseline ARVC calculator was assumed to be at random and imputed using multiple imputation with chained equations[14]. The final model included all predictors included in the ARVC risk calculator together with VA outcome and a cumulative baseline hazard estimation. A total of 20 imputed datasets were generated using 20 iterations each, and the final estimates were combined using Rubin's rule[15]. Categorical variables were summarized as frequencies (%) and compared using proportional z-test. Continuous variables were presented as mean ± standard deviation or median [interquartile range (IQR)], and compared using the independent sample Students t-test or the Mann–Whitney U-test, as appropriate.

For patients with known risk factor values at both the time of diagnosis and at least 3-years of follow-up, Wilcoxon signed rank tests were used to assess differences between risk factor values at the two time points. For patients with >1 repeat risk factor assessment, the value from closest to 5-years of follow-up was selected. Follow-up duration was calculated from the date of diagnosis to the date of composite outcome occurrence or censoring (defined as death from any other cause, heart transplantation, or the most recent follow-up visit). Survival curves were estimated using the Kaplan–Meier method. The strengths of associations between risk factor variables and VA events were reported as hazard ratios derived from Cox proportional hazards modeling of baseline risk factors and from unrestricted Cox regression analysis of all available longitudinal risk factor data.

The longitudinal performances of the three methods for estimating 5-year VA risk were compared by generating time-dependent receiver operator characteristics and calculating the area under these curves (ROC-AUC) for each follow-up time between time of diagnosis and 5-years [16]; error was reported with 95% confidence intervals and curves were smoothed to facilitate visual interpretation using locally weighted scatterplot smoothing with a weighting fraction of 0.2.

Calibration was assessed by calculating the mean risk predictions for low (0-10%), intermediate (10-25%), and high (>25%) risk patients as assessed by the modified ARVC risk calculator at time of diagnosis, and comparing to mean observed risk as estimated by the Kaplan-Meier method in these risk groups. Differences in predicted versus observed risk (miscalibration) were assessed using empiric exponential decay functions (**Supplemental Methods III**) for both the overall cohort and each of the three risk groups.

RESULTS:

Study Population

The study included 408 patients, of whom 146 were from the Netherlands ACM registry (36%) and 262 were from the Johns Hopkins ARVC registry (64%). Patient characteristics by registry are shown in **Supplemental Table II**. A minority were male (n=164, 40%). The age at ARVC diagnosis was 37±15 years and about two-thirds had symptoms attributable to ARVC at the time of diagnosis (n=232, 64%). Most patients were identified as having pathogenic genetic variants (n=298, 74%), most commonly in *PKP2* (n=197, 49%). More than half the patients were probands (n=240, 58.8%). **Table 1** summarizes other clinical and demographic characteristics.

Table 1: *Baseline characteristics of ARVC patients at the time of ARVC diagnosis. Continuous variables are presented as mean ± standard deviation or median [IQR], as appropriate.*

Variable	All Patients (n=408)	Absence of VA event (276)	Occurrence of VA event (132)	p value
Age at Diagnosis (n=408)	37 (±15.1)	38 (±15.8)	33 (±12.6)	<0.001
Male Sex (n=408)	164 (40.2%)	96 (34.8%)	68 (51.5%)	0.001
Caucasian Race (n=407)	397 (97.5%)	269 (97.5%)	128 (97.7%)	0.881
Proband (n=408)	240 (58.8%)	135 (48.9%)	105 (79.5%)	<0.001
Pathogenic / likely pathogenic variant (n=405)	298 (73.6%)	211 (77.0%)	87 (66.4%)	0.024
PKP2	197 (48.6%)	133 (48.5%)	64 (48.9%)	0.953
DSP	13 (3.2%)	10 (3.6%)	3 (2.3%)	0.468
DSG2	11 (2.7%)	5 (1.8%)	6 (4.6%)	0.111
PLN	27 (6.7%)	20 (7.3%)	7 (5.3%)	0.46

Other	13 (3.2%)	7 (2.6%)	6 (4.6%)	0.279
Symptoms* (n=361)	232 (64.3%)	131 (55.3%)	101 (81.5%)	<0.001
History of Cardiac Syncope (n=408)	77 (18.9%)	39 (14.1%)	38 (28.8%)	<0.001
Anterior T-wave inversions (n=398)	3 [2.0; 4.0]	3 [1.0; 4.0]	3 [3.0; 4.0]	<0.001
Inferior T-wave inversions (n=387)	0 [0.0; 1.0]	0 [0.0; 1.0]	0 [0.0; 1.0]	0.028
Total T-wave inversions (ant.+inf.) (n=387)	3 [2.0; 5.0]	3 [2.0; 4.0]	4 [3.0; 5.0]	<0.001
24 hr. PVC count (n=343)	1186 [361; 4095]	860 [183; 2751]	2879 [1151; 60785]	<0.001
Presence of NSVT (n=370)	195 (52.7%)	114 (44.4%)	81 (71.7%)	<0.001
RVEF (%) (n=348)	44 (±10.1)	45 (±8.8)	40 (±11.6)	<0.001
LVEF (%) (n=355)	58 (±8.0)	58 (±7.9)	57 (±8.3)	0.304
ICD at any point (n=407)	277 (68.1%)	150 (54.5%)	127 (96.2%)	<0.001
ICD prior to dx.	18 (4.4%)	16 (5.8%)	2 (1.5%)	0.049
ICD within 6 mo. of dx.	129 (31.6%)	59 (21.4%)	70 (53.0%)	<0.001
ICD arrhythmia monitoring zone cycle length (ms)	350 [323, 400]	350 [328, 375]	351 [322, 400]	0.520
ICD arrhythmia treatment zone cycle length (ms)	300 [286, 320]	300 [285, 316]	300 [289, 333]	0.017
Baseline anti-arrhythmic prescription (n=391)	59 (15.1%)	36 (13.5%)	23 (18.4%)	0.21
Amiodarone prescription	8 (2%)	5 (2%)	3 (2%)	0.753
Sotalol prescription	45 (11%)	27 (10%)	18 (14%)	0.245
Baseline beta-blocker prescription (n=392)	153 (39.0%)	96 (36.0%)	57 (45.6%)	0.068
ARVC calculator predicted 5-year VA risk (%)	29 (±23%)	21 (±19%)	45 (±23%)	<0.001
Observed 5-year VA risk (%)	29% [95%CI: 24, 34]		---	---

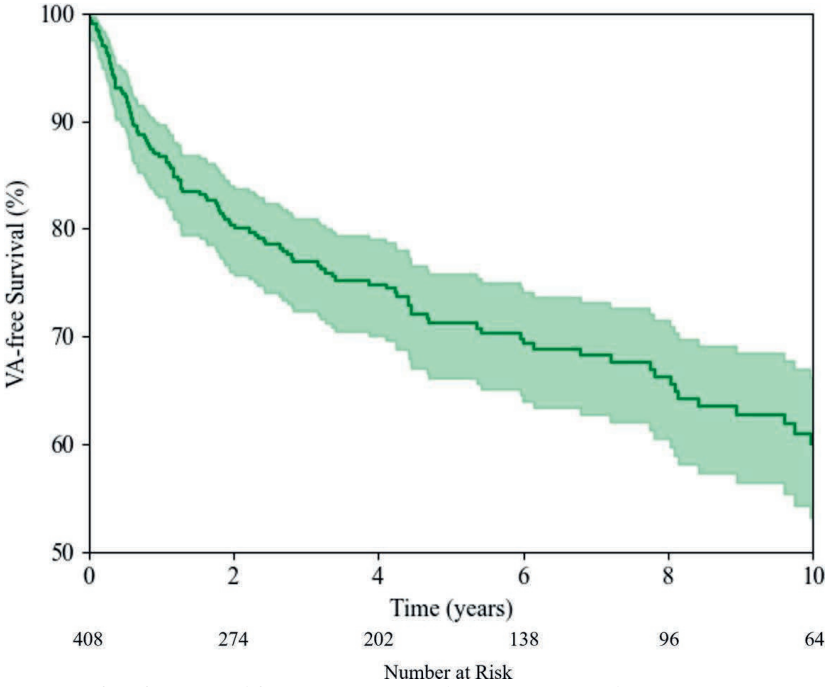
Overall, 282 patients (69%) had complete baseline risk factor data allowing for estimation of the ARVC calculator 5-year VA risk. Missing data occurred for five of the eight predictors: NSVT (n=38, 9.3%), PVC count (n=65, 15.9%), number of TWI (n=21, 5.1%), RVEF (n=60, 14.7%). After imputation, mean 5-year VA risk was estimated at 29% [95% CI: 24, 34%] using the ARVC risk calculator.

Outcomes

During median follow-up of 5.2 [IQR: 2.8, 9.6] years, 132 (32%) patients experienced the composite VA outcome at a rate of 6.3 events per 100 patient-years. **Figure 1** shows the cumulative

VA free survival. Events occurred throughout follow-up, with a cumulative VA free survival at 5-years of 71.3% [95% CI: 75.8, 66.1].

Figure 1:



Kaplan-Meier estimate of VA free survival for ARVC patients without prior sustained VA.

Of these events, 87 (66%) were ICD interventions, including either appropriate shock or anti-tachycardic pacing, and had median cycle length of 270 ms [IQR: 235, 300]. Rapid sustained VAs (VT with cycle length <240 ms, SCA, or SCD) occurred in 41 (10.0%) patients during follow-up at a rate of 1.6 events per 100 patient-years. At last follow-up, 6 (1.5%) patients had died and 10 (2.5%) had undergone heart transplantation. Of these alternative outcomes, 0 deaths and 1 transplant occurred without prior VA event; competing-risk sensitivity analysis was performed and did not impact results.

Longitudinal Predictive Variables

Table 2 details the number and timing of VA risk factor re-evaluations during clinical follow-up, the distributions of which are presented as histograms in **Supplemental Figure I** and **Supplemental Figure II.**

Table 2: *Number and timing of VA risk predictor reevaluations during clinical follow-up.*

Evaluation	Number of Pts. w/Additional Evals.	Number of additional Eval. (per patient)	Time of evaluation [IQR]
Ambulatory cardiac monitor (PVC, NSVT)	294	951 (2.3)	3.0 [1.0, 6.8]
ECG (TWI)	344	1,429 (3.5)	3.5 [1.1, 7.6]
Echo (RV function)	173	483 (1.2)	5.1 [2.1, 9.6]
Echo (LVEF)	251	735 (1.8)	4.4 [1.7, 8.9]
Medication review	104	220 (0.5)	3.6 [0.6, 8.5]
Exercise histories	102	102 (0.25)	4.7 [1.9, 9.0]

Average changes in risk factor values between time of ARVC diagnosis and 5-year follow-up are presented in **Table 3**.

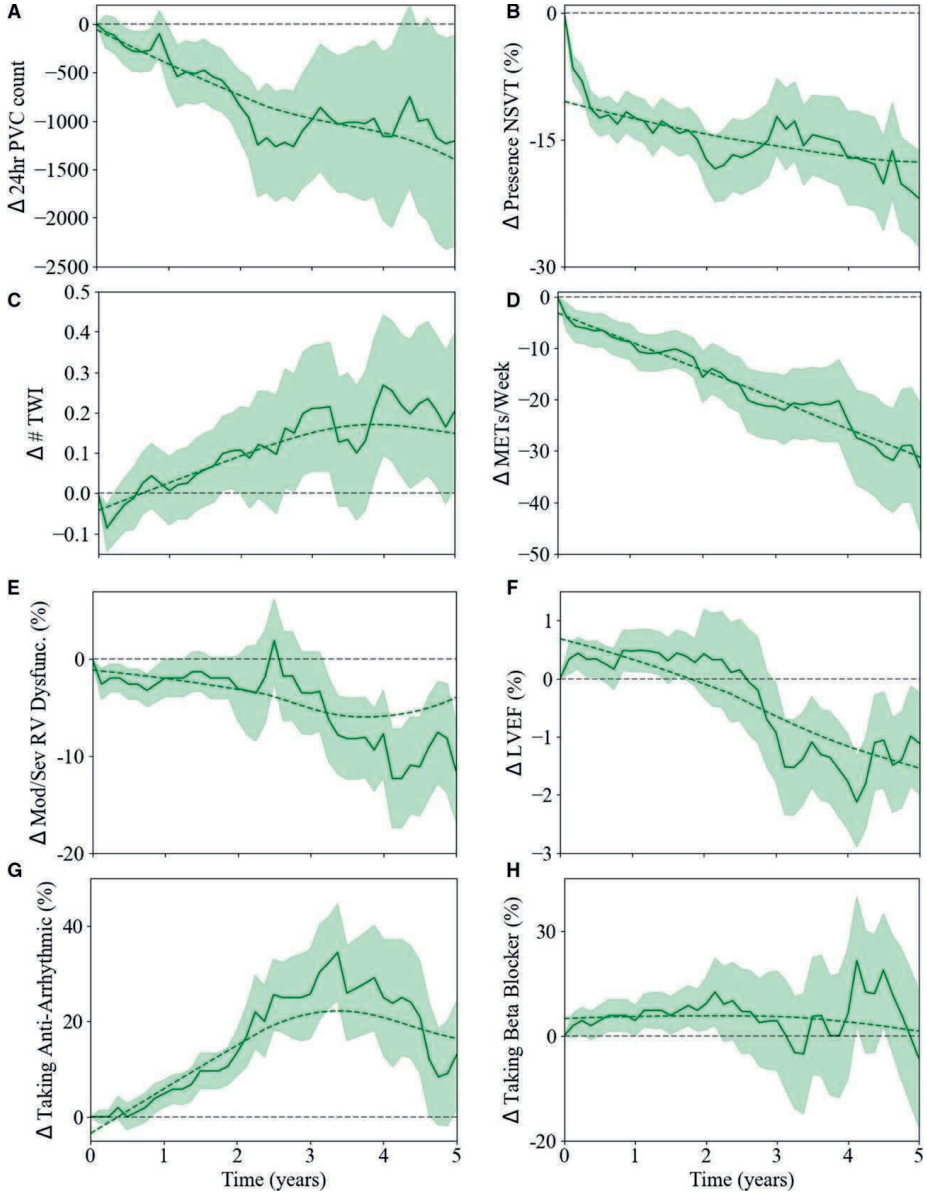
Table 3:

	Change from Diagnosis to >5 years follow-up	p-value
ARVC risk calculator variables		
Log(24 hour PVC count) (n=112)	-0.64±2.5	0.009
Presence of NSVT (n=122)	-14%	0.006
Number of TWI (n=161)	0.0 [-1.0, 1.0]	0.456
Presence of RV dysfunction (mod/sev) (n=102)	+6%	0.181
Other Risk predictors		
LVEF (%) (n=150)	-2.2±7.5	<0.001
Anti-arrhythmic medication prescribed (n=49)	+16%	0.044
Beta blocker prescribed (n=49)	+10%	0.255
Exercise (MET*hr/week) (n=46)	-4 [-42, 7]	0.016

On repeat ambulatory cardiac monitoring assessment, the prevalence of NSVT decreased by 14% and the burden of PVCs decreased by an average of 1,200 PVC per 24 hours. There was a nonsignificant trend towards increased prevalence of moderate to severe RV dysfunction. Sensitivity analysis was performed looking at changes between individual RV functional categories (e.g. normal, mild, moderate, and severe dysfunction) and likewise did not reveal significant changes. There was a small but statistically significant 2% decrease in LVEF. There were no significant changes in the number of TWI on repeat ECG. There was a significant increase of 16% in the prescription rates of anti-arrhythmic medications, but no change in the rates of beta blocker

prescriptions. On average, patients decreased their exercise by 4 MET*hr/week. **Figure 2** shows the longitudinal trends of the changes in these variables.

Figure 2



Longitudinal trends in predictors of VA events, presented as the change in predictor value at follow-up relative to time of ARVC diagnosis: A) log of 24-hour PVC burden, B) presence of NSVT on cardiac ambulatory monitoring, C) number of TWI in precordial and inferior leads, D) the extent of strenuous exercise per week, E) presence of moderate or severe RV dysfunction from echocardiography, F) LVEF from echocardiography, G) anti-arrhythmic prescription, and H) beta blocker prescriptions

Longitudinal Risk Prediction

Associations between individual elements of the modified ARVC risk calculator and VA events are presented in **Table 4.** The C-statistic of the modified ARVC risk calculator for 5-year VA events was 0.76±0.02 and was similar to that of the original ARVC risk calculator (C-statistic 0.78).

Table4

	Cox regression using baseline variables Hazard ratios [95% CI]				**Time varying Cox regression Hazard ratios [95% CI]**			
Variable	**Univariable**	**p**	**Multivariable**	**p**	**Univariable**	**p**	**Multivariable**	**p-**
Age (years) (per year)	0.983 [0.972; 0.995]	0.004	0.978 [0.966; 0.989]	<0.001	0.983 [0.972; 0.995]	0.004	0.983 [0.971; 0.994]	0.003
Male sex (vs. female)	1.843 [1.307; 2.600]	<0.001	1.746 [1.234; 2.471]	0.002	1.843 [1.307; 2.600]	<0.001	1.730 [1.222; 2.449]	0.002
NSVT presence	3.653 [2.434; 5.484]	<0.001	2.126 [1.350; 3.347]	0.001	3.012 [2.082; 4.356]	<0.001	1.758 [1.165; 2.652]	0.007
History of cardiac syncope	2.197 [1.504; 3.209]	<0.001	1.554 [1.050; 2.298]	0.027	2.470 [1.713; 3.562]	<0.001	1.794 [1.232; 2.612]	0.002
# TWI (per lead)	1.199 [1.107; 1.297]	<0.001	1.100 [1.004; 1.206]	0.04	1.176 [1.090; 1.269]	<0.001	1.079 [0.994; 1.171]	0.071
log(24 hour PVC count)	1.536 [1.366; 1.729]	<0.001	1.321 [1.156; 1.510]	<0.001	1.381 [1.243; 1.533]	<0.001	1.207 [1.080; 1.348]	0.001
Presence of mod./sev. RV dysfunction	2.745 [1.922; 3.920]	<0.001	1.421 [0.968; 2.084]	0.073	2.961 [2.076; 4.225]	<0.001	1.807 [1.239; 2.637]	0.002

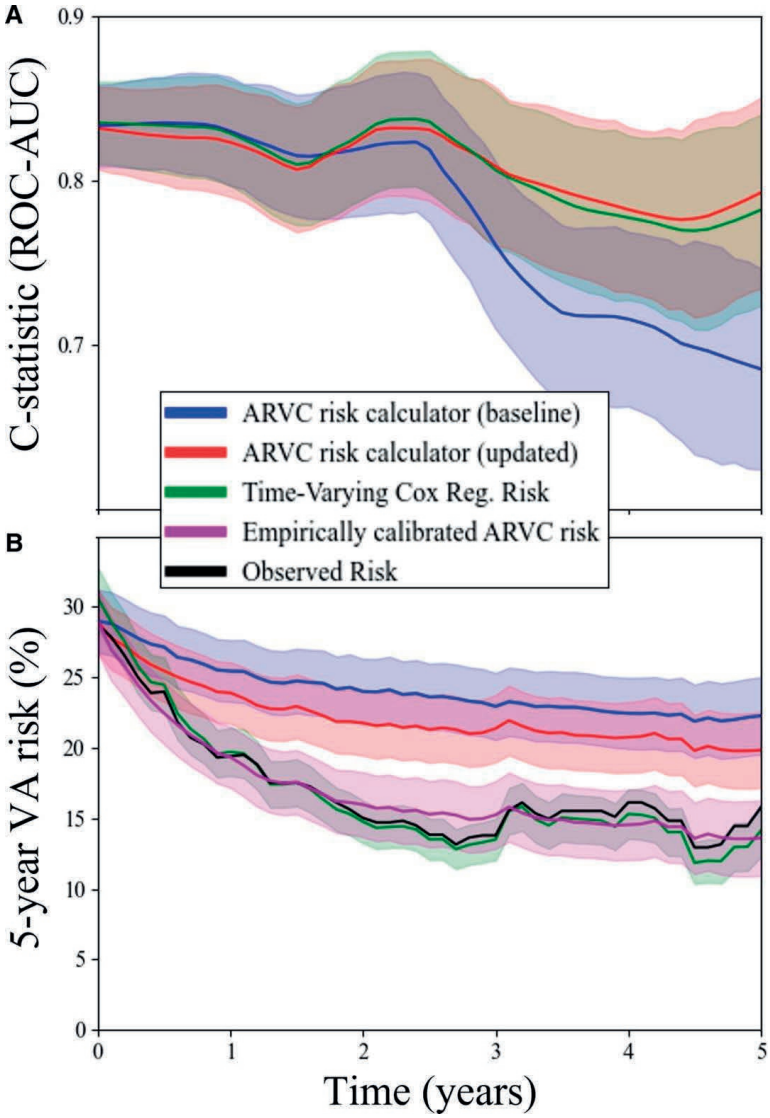
Associations between clinical risk factors included in the modified ARVC risk calculator and 5-year VA event risk. Hazard ratios are presented with 95% confidence intervals.

Figure 3a presents longitudinal trends in model discrimination of 5-year VA events for the 3 risk prediction methods (baseline ARVC risk calculator, updated ARVC risk calculator, and time-

varying Cox regression). As shown in **Figure 3a**, the ability to discriminate VA event risk decreased for the baseline ARVC risk calculator after approximately 3-years and the C-statistic decreased from 0.83±0.03 at time of diagnosis to 0.69±0.06 at 5-years, while the updated ARVC risk calculator and time-varying Cox regression risk remained relatively stable out to 5-years (C-statistics of 0.83±0.03 to 0.79±0.06 and 0.84±0.03 to 0.78±0.06, respectively).

Mean VA risk predictions from the three models are shown in **Figure 3b**, where they are compared to observed VA risk. Observed 5-year risk decreased from 29% to 16% between the time of initial ARVC diagnosis and at 5-year follow-up. While all models showed a decrease in predicted 5-year VA risk at 5-year follow-up, these decreases were smaller in magnitude for both baseline ARVC risk calculator (29% decreasing to 22%) and updated ARVC risk calculator (29% decreasing to 20%). The time-varying Cox regression risk predictions (31% decreasing to 14%) more closely matched the observed drop in 5-year risk. Risk predictions from the updated ARVC risk calculator were recalibrated using an empiric exponential decay function, resulting in close approximation of observed risk (29% decreasing to 14%). The average risk discrepancy estimated using this calibration model was +6%. Risk discrepancies in the low, intermediate, and high-risk groups were +2%, +9%, and +13%, respectively. Subgroup calibration plots are shown in **Supplemental Figure III,** and details of empiric calibration models are presented in **Supplemental Table III**.

Figure 3



A) *Longitudinal changes in model discrimination for (blue) the baseline ARVC risk calculator, (red) the updated ARVC risk calculator, and (green) time-varying Cox regression risk. Plots are shown with LOWESS smoothing and standard errors of the mean.* ***B)*** *Longitudinal calibration between predictions made by (blue) baseline ARVC risk calculator, (red) updated ARVC risk calculator, (green) time-varying cox regression risk, compared to observed risk (black). The updated ARVC risk calculator risk was recalibrated using an empiric exponential decay function (magenta). Here observed risk is shown with 95% confidence intervals, and model predictions are shown with standard errors of the mean.*

DISCUSSION:

In this study we leveraged a large, deeply phenotyped, multicenter cohort of ARVC patients with long-term follow-up to characterize how VA risk factors change over time and to define how these changes can be incorporated into models for longitudinal VA risk prediction. Our findings shed important new insights into the dynamic nature of the disease course of ARVC following initial diagnosis. In particular, we demonstrated the importance of changes in ventricular ectopy, with both prevalence of NSVT and PVC burden acting as independent risk factors for VA events that decrease during follow-up. Overall likelihood of primary VA events likewise decreased over time. Applying the same baseline ARVC risk calculator prediction to follow-up evaluations resulted in decreasing VA risk discrimination after 3 years. However, this decrement was negated by updating the ARVC risk calculator prediction with changes in risk factor values (i.e. assessing 5-year VA risk using the ARVC risk calculator and the most recent set of available risk factor data). Mean risk for initial VA event during the subsequent 5-year period was overestimated by an average of +6% compared to both observed risk, though this overestimate was smaller in low-risk patients. We created a time-varying Cox regression model for predicting 5-year VA risk that maintained excellent discrimination and accuracy at 5-year follow-up.

Comparison to other study findings

While there have been a handful of studies reporting longitudinal changes in individual ARVC risk factors, our study represents the first examination of how these risk factors change in concert with one another. Similar to our findings, one recent observational study examining patients with multiple Holter monitors found that the average burden of PVC decreased after initial diagnosis[10]. This study likewise demonstrated the importance of changes in PVC count, with both presence of NSVT and increase in PVC burden independently identifying increased risk for VA events in the year following assessment. Cappelletto et al. likewise found that both NSVT and PVC burden decreased progressively at both 2-year and 8-year follow-up in their cohort of patients with repeat Holter monitoring [17], and that NSVT remained an important independent risk factor for VA

at follow-up. It is unclear whether these changes are part of the natural disease course in ARVC, or if decreased ventricular ectopy is the result of initiating pharmacologic therapy and lifestyle modification. It is also plausible that the observed improvements in electrophysiologic properties may be exaggerated due to selection bias, as both PVC count and NSVT are important arrhythmic components of the ARVC diagnostic criteria.

For patients with repeat echocardiographic assessment, we found that cardiac function was stable between ARVC diagnosis and 5-year follow-up. On average, patients did not have progressive RV dysfunction during that period. While patients did demonstrate a statistically significant 2% decrease in LVEF, this small change is unlikely to be clinically significant. These findings are consistent with other studies that have looked at changes in cardiac function in ARVC over time. In a smaller study of ARVC patients with serial echocardiograms, Malik et al. found small but significant decreases in LVEF without significant changes in RV fractional area change over a similar time frame[18]. Contrasting this, Taha et al. found that RV fractional area change decreased by 5% over 7-year follow-up of ARVC patients with serial imaging[19]. Kalantarian et al. found that about a quarter of ARVC patients had a drop of at least 10% in RV fractional area change over 10-year follow-up [11]. Thus, significant functional cardiac changes in ARVC seem to occur over longer time scales (> 5 years) than our present study was able to examine. It is also possible that our evaluation of RV function as a dichotomous rather than continuous variable (e.g. fractional area change or ejection fraction) may have overlooked more subtle, early progression of RV dysfunction. We likewise did not explore more sensitive markers such as echocardiographic or CMR based RV strain that have been shown to be associated with progression of RV dysfunction[18]. However, we did not find that substitution of RVEF with a categorical definition of RV dysfunction negatively impacted the ARVC risk calculator's ability to discriminate VA risk, suggesting that these early changes are less important for predicting incident VA. This is consistent with prior studies showing that RV strain did not add incremental value to prediction of VA over broader assessments of RV dysfunction[20].

We also found that the number of TWI on ECG was relatively stable out to 5-years of follow-up. These findings are consistent with prior work demonstrating that while TWI in both the inferior and precordial leads are common, they change little by around 5-years of follow-up [21, 22]. In contrast, studies examining longer-term follow-up with serial ECGs out to 10-years have demonstrated increased numbers of ECG leads with TWI [11, 23, 24]. As with cardiac functional changes, these findings suggest that the progression of ECG changes, and thus the electrophysiologic and structural changes they reflect, likely change over longer time spans (>5 years) than the present study was able to examine.

Longitudinal trends in VA risk and risk prediction

We found that average risk for first sustained VA event decreased by nearly half (absolute risk reduction of 13%) between initial evaluation and 5-year clinical follow-up (**Figure 3**, black line). This may in part be due to the selection bias inherent to this type of analysis. Those high-risk patients present in the initial cohort who go on to have VA events are by definition no longer at risk for a first VA event. They are thus removed from the pool of patients for whom risk of initial VA events are subsequently assessed. This is reflected by the negative trend in risk predicted by the baseline ARVC risk calculator (**Figure 3,** blue lines) and accounts for an approximately 7% decrease in average risk by 5-years. This only partially explains the total 13% decrease in observed risk, however. The discrimination of the baseline ARVC risk calculator also drops off significantly after 3-years, suggesting that there is also heterogeneity in the way that individual patient risk changes over time. Accounting for changing patient characteristics by recalculating the predicted risk with the most recent set of risk factor data results in significantly improved discrimination of VA likelihood (**Figure 3**, red lines) but a persistent overestimation of mean risk (+6% at 5-year follow-up). In contrast, the time-varying Cox-regression model for risk prediction had both good discrimination and well calibrated mean risk (**Figure 3**, green lines). This model takes advantage of complete knowledge of the baseline hazard function (e.g. the instantaneous VA risk at all follow-up times for a patient with null risk factors), and thus incorporates empiric changes to risk that exceed

those accounted for by the included VA risk factors. Similarly, we were able to recalibrate the updated ARVC risk calculator predictions using an empiric exponential decay function (**Figure 3**, magenta lines), which resulted in both excellent discrimination and closely calibrated mean risk. Thus, there appear to be three distinct sources of decreasing VA risk: survivorship bias, improving risk factors included within the ARVC risk calculator (age, NSVT, and PVC count), and additional risk modifiers that are currently unaccounted for by the ARVC risk calculator but that do not impact risk discrimination.

Two risk modifiers that may decrease longitudinal VA risk but are not included in the ARVC risk calculator are reductions in exercise and initiation of medical therapy. We found that patients significantly reduced the amount and intensity of their exercise between initial diagnosis and 5-year clinical follow-up (**Figure 2**, panel D). Prior studies have shown that competitive sports activity is associated with as much as a 5-fold increase in risk for SCD in young adults [25 26], and that this association is dose-dependent [27, 28]. Even recreational sports contribute significantly to risk of VA and SCD [29]. In this context, our results support decreasing exercise as a plausible mechanism for reducing risk for VA. We also found that patients in our cohort were more likely to be prescribed anti-arrhythmic medications at 5-year follow-up compared to at the time of initial diagnosis (**Figure 2**, panel G). While evidence for the use of anti-arrhythmic medications in ARVC is mixed [30], observational data suggests that these medications, particularly amiodarone and sotalol, may reduce the rate of VA events in patients with high burdens of PVC and NSVT [31-34]. We did not find that rates of beta-blocker prescriptions changed significantly between initial ARVC diagnosis and 5-year follow-up. This may be because of the moderately high rates (~40%) of baseline beta blocker prescriptions, and the lack of strong evidence supporting their efficacy in isolated right sided dysfunction or for prevention of VA events[26]. In addition, it has been hypothesized that episodes of acute inflammation elicited by environmental triggers may play a role in modulating disease progression[35]. As inflammation increases both VA risk and symptom burden, it follows that

ARVC diagnosis is most likely to be made during an inflammatory episode, thus leading to the observed pattern of heightened initial VA risk followed by risk attenuation as the episode recedes.

Clinical Implications

Our time-varying Cox regression model provided a combination of strong discrimination and accurate VA risk prediction. However, its clinical use would likely be cumbersome due to the need for providers to enter a significant quantity of risk factor data in order to generate risk predictions. Ultimately this could be achieved via integration into electronic health records systems. Alternatively, our findings suggest that the ARVC risk calculator remains a useful clinical tool for discriminating between low- and high-risk patients during follow-up evaluation, provided that predictions are made using updated risk factor data. Predictions made by the ARVC risk calculator overestimate the observed risk at follow-up evaluations, the average magnitude of which was +6%. This overestimation is smaller (+2%) in patients with low baseline risk and larger in patients with high baseline risk (+13%) (**Supplemental Table III**). Since those patients at low baseline risk are least likely to have ICD placement at time of ARVC diagnosis, the updated ARVC risk calculator therefore performed best in the population for whom longitudinal VA risk reassessment was most relevant.

Additionally, we present a modified version of the ARVC risk calculator which makes use of a dichotomous RV dysfunction variable, rather than continuous RVEF. This modification did not decrease the model's discrimination in this cohort and has the added benefit of eliminating the score's reliance on CMR imaging data which may be unavailable at follow-up (particularly after ICD implantation) or granular RV fractional area change which may not be routinely available in clinical echocardiograms. External validation will also be required before this modified risk prediction tool should be used clinically.

Finally, our findings are consistent with the hypothesis that reduction in exercise and initiation of anti-arrhythmic medications may help to reduce the likelihood of VA events. While Bosman et al examined the incremental value of adding exercise to the ARVC risk calculator and

found no improvement in VA risk prediction, their analyses were restricted to risk prediction at the time of initial ARVC diagnosis[28]. It is possible that reducing exercise and initiating anti-arrhythmic medications may be important for improving individualized, longitudinal risk predictions. That said, in one small cohort of athletic ARVC patients, ARVC risk calculator predictions also seemed to hold despite clinical detraining [36]. Further analyses of cohorts with more complete exercise history and medication review data are therefore needed to clarify the incremental value of these variables in longitudinal VA risk prediction. Regardless, the updated ARVC risk calculator had excellent discrimination without inclusion of either exercise or medication data.

Limitations:

We acknowledge the observational nature of this study as a limitation. All longitudinal reassessments of risk predictors were obtained at the discretion of the local clinicians introducing possible observation bias. However, this observation bias most likely takes the form of increased surveillance in high-risk patients and those with clinical symptoms, which represent the population for whom VA risk prediction is of most relevance. Additionally, while many repeat diagnostic tests were available during follow-up, the number of patients for whom complete exercise histories and longitudinal medication reviews were available represent a small fraction of the overall cohort, and may have therefore increased the risk of type 2 error (e.g. our failure to detect change in beta blocker prescription rate) and/or be less representative of the full cohort. To confirm our hypotheses that the differences between observed VA event rates and uncalibrated ARVC risk predictions are due to these risk modifiers, further studies with more complete exercise and medication review data should be performed. Finally, our study population was drawn from tertiary, academic centers from North America and Northern Europe which may have created a referral bias that could lead to overestimation of VA risk in a community-derived population. External validation of our model for longitudinal VA risk assessment is essential to confirm its clinical utility. Additionally, as in the original ARVC risk calculator, we used a surrogate composite endpoint that included appropriate ICD therapy to infer risk of SCD. While clinically recognized as significant arrhythmic events, ICD

therapies are an imperfect substitute for SCD[37]. As a further limitation of our multi-center, longitudinal registry-based study of a rare disease, we do not have granular data regarding the breakdown of these ICD therapies into anti-tachycardic pacing versus appropriate shock, or of the programmed detection times for therapies.

Conclusions:

In the present study, we leveraged a well-characterized, international multi-center cohort of ARVC patients with long-term clinical follow-up to explore the ways in which risk factors for VA change over time, how these changing risk factors impact overall rates of sustained VA, and how well current risk assessment tools perform on serial evaluation. On average, we found that ventricular ectopy including both burden of PVCs and prevalence of NSVT decreased significantly between time of diagnosis and 5-year follow-up, while structural and functional risk factors including RV function and number of TWI on ECG remained largely static. We found that updating the ARVC risk prediction using the most recent set of VA risk factors was important in maintaining discrimination during follow-up. Additionally, observed 5-year VA risk decreased quickly relative to predicted risk, suggesting the influence of risk modifiers that are not explicitly included in the ARVC risk calculator. Mean VA risk was overestimated by +6% at 5-year follow-up, and this overestimation should be accounted for when providing clinical risk assessments.

Funding: This research was funded by multiple generous sources, including the Johns Hopkins Cardiology NIH T32HL007227 training grant (RTC), the Leonie-Wild Foundation, the Wilton W. Webster Fellowship from the Heart Rhythm Society (AG), the Netherlands Heart Foundation grant 2015T058 and UMC Utrecht Fellowship Clinical Research Talent, and the Young Talent Program CVON2012-10 PREDICT (ASJMtR) and PREDICT2 (AAMW and JPvT). We acknowledge the support from the Netherlands Cardiovascular Research Initiative, an initiative with support of the Netherlands Heart Foundation, grant nos.: CVON2012-10 PREDICT, CVON2015-12 eDETECT. The Netherlands ACM Registry is supported by the Netherlands Heart Institute (project 06901). The Johns Hopkins ARVC Program is supported by the Leyla Erkan Family Fund for ARVD

Research, the Dr. Francis P. Chiramonte Private Foundation, the Dr. Satish, Rupal, and Robin Shah ARVD Fund at Johns Hopkins, the Bogle Foundation, the Healing Hearts Foundation, the Campanella family, the Patrick J. Harrison Family, the Peter French Memorial Foundation, and the Wilmerding Endowments.

Disclosures:

Dr. Calkins consults for Medtronic Inc., Biosense Webster, Pfizer, StrideBio, and Abbott. Ms. Murray consults for MyGeneCounsel. Dr. James consults for Pfizer, Inc, Tenaya Inc., and StrideBio, Inc. Dr. Calkins, Dr. James, and Ms. Tichnell receive research support from Boston Scientific Corp. Dr. Tandri receives research support from Abbott. Dr. Yap consults for Boston Scientific Corp. Dr Wilde consults for LQTherapeutics and ARMGO.

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Supplemental Materials

Methods I: Derivation of the modified ARVC risk calculator
Due to high rates of primary prevention ICD placement and the subsequent low incidence of repeat CMR during follow-up, the predominantly CMR derived RVEF variable was replaced with an alternative categorical definition of RV function. Categorical RV function (here defined as normal, mild, moderate, or severe dysfunction) was adjudicated based upon qualitative echocardiographic assessments of the RV, RV fractional area change on echocardiography, or RV ejection fraction on CMR, as available (**Definitions in table below**). A Cox proportional hazards model was refit to the cohort after substitution of continuous RVEF with presence of moderate or severe RV dysfunction as a dichotomous variable. Sensitivity analysis demonstrated stable discrimination of baseline 5-year risk between the original and modified ARVC calculator (C-statistic 0.772 versus 0.774).
RV function category definitions

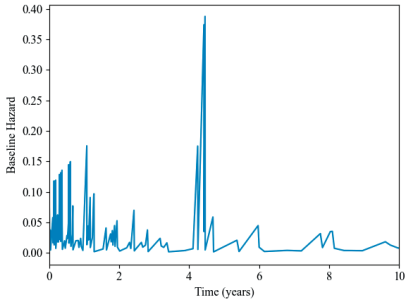
RV dysfunction	RV fractional area change (echocardiography)	RV ejection fraction (CMR)
Normal	>40%	>45%
Mild	33-40%	40-45%
Moderate	20-33%	30-40%
Severe	<20%	<30%

Methods II: Time-varying Cox Regression Risk Prediction
An unrestricted (non-proportional) Cox regression model was fit to predictor variables included in the modified ARVC risk calculator, thus allowing for incorporation of temporal trends in these variables (time-varying Cox risk). This model was used to estimate subsequent 5-year VA risk for the i^{th} patient from an arbitrary follow-up time (t_{fu}) according to **Equation I**.

$$\text{(I)} \qquad P_i(Sustained\ VA\ in\ subsequent\ 5\ years) = 1 - \exp\left(-\int_0^{t_{fu}+5yr} \exp\left(\sum_{j=1}^{p} \beta_j x_{ji}(u)\right) * h_0(u)\mathrm{d}u\right)$$

Here, β_j is the fitted beta coefficient of the j^{th} risk factor, $x_{ji}(u)$ is the trend in the j^{th} risk factor for the i^{th} patient, and $h_0(t)$ is the baseline hazard function obtained from fitting the Cox regression model with p time dependent variables[38]. Here, the beta coefficients and baseline hazard function are given in the table and figure below, respectively.

Risk factor	Beta Coefficient
Age (years)	-0.017
Male sex (yes/no)	0.548
NSVT (yes/no)	0.564
Cardiac Syncope (yes/no)	0.584
Number of TWI	0.076
ln(PVC burden/24hrs)	0.188
Moderate/Severe RV dysfunction (yes/no)	0.592



Methods III: Empiric risk calibration

We evaluated both observed and predicted VA risk as presented in Figure 3b of the manuscript. Based on visual inspection demonstrating short term divergence followed by approximately parallel rate of VA incidence, an exponential decay function was selected for empiric assessment of observed versus predicted risk discrepancy (**Equation II**).

$$\text{(II)} \quad \text{Recalibrated Risk}(t_{fu}) = \text{Risk Prediction}(t_{fu}) - \gamma * (1 - \exp(-\tau * t_{fu}))$$

Here, γ reflects the difference in observed and predicted risk at $t_{fu} \to \infty$, and τ is the time constant reflecting how rapidly this difference is reached (small values reflect slow approach, large values reflect rapid approach).

Table I: Standard list of definitions for local data collection.

Variable Name	Description and definition Units
DEMOGRAPHIC PARAMETERS	
Site	Site of Enrollment.
Age at diagnosis	Age at which definite ARVC was attained according to 2010 Task force criteria (TFC): i. 2 major criteria (from 2 different categories) ii. ii. 1 major and 2 minor criteria (from 3 different categories) iii. iii. 4 minor criteria (from 4 different categories) Days
Strenuous Exercise before diagnosis	Participation in strenuous exercise before Diagnosis (ACC AHA class C). Individual who participated in sports with a high dynamic demand (>70% max O2), as defined by the 36th Bethesda Conference Classification of Sports, at vigorous intensity at any point in their life (prior to one year after dx/or first event). Yes = 1, No = 0
GENETIC PARAMETERS	
Sex	Chromosomal sex of the patient. Male = 1, Female = 0
Pedigree	Proband defined as first affected family member seeking medical attention for ARVC, in whom the diagnosis was confirmed (i.e. an individual ascertained independently of family history). Proband = 1, Family Member = 2
Race	Ethnicity of the patient Caucasian = 1, Black = 2, Asian = 3
Mutation	Pathogenic mutation associated with ARVC detected by genetic screening. Nonsense, frameshift, splice site mutations and exon deletions are considered proven pathogenic unless previously identified as polymorphism. Missense mutations are considered pathogenic when 1) Minor allele frequency in Exome sequencing project was ≤0.05%, and 2) in silico prediction programs predicted the variant to affect protein function by score <0.02 (SIFT) and >0.900 (Polyphen2). Mutations in desmosomal genes and non-desmosomal genes (PLN) will be considered pathogenic Yes = 1, No = 0
Gene	Gene with identified mutation. PKP2 = 1, DSP=2, DSG2 = 3, DSC2 = 4, JUP = 5, TMEM43 = 6, PLN = 7, CH/HO/DG (CH: compound heterozygous mutations; DG: digenic mutations; HO: homozygous mutations) = 8, Other = 9
Amino Acid	Amino acid change(s). free text
DNA Change	Nucleotide changes (cDNA). free text
Genotype	Gene with mutation and base pair chain (cDNA genotype). Text
SYMPTOMATIC PARAMETERS	
Symptoms at Diagnosis	Presence of symptoms associated with ARVC at diagnosis as reported in the medical notes (prior to one year after dx/or first event) Present = 1, Absent = 0

Recent Cardiac Syncope	Transient loss of consciousness and postural tone with spontaneous recovery with arrhythmic mechanism likely at diagnosis occurring within 6 months of initial diagnosis. This thus excludes syncope of vaso-vagal etiology (prior to one year after dx/or first event)
Age at Cardiac Syncope	Age at the time of observed cardiac syncope event. Days
MEDICATION PARAMETERS	
Anti-arrhythmics at time of diagnosis	Patients is prescribed an anti-arrhythmic medication at the time of diagnosis. None = 0, Amiodarone = 1, Sotalol = 2, Class 1C AA = 3, Dofetilide = 4, Mexiletine =5, Other = 6
Anti-Arrhythmics at time of first VA event	List of all anti-arrhythmic medication taken at time of first event or censoring (list sotalol here). Text
Beta-blockers at time of diagnosis	Betablockers (excluding sotalol) taken at diagnosis Yes = 1, No = 0
Beta-blockers at time of first VA event	Betablockers (excluding sotalol) taken at time of first event or censoring Yes = 1, No = 0
ELECTROCARDIOGRAPHIC PARAMETERS	
ECG at diagnosis	ECG performed at diagnosis (prior to one year after dx/or first event) Yes = 1, No = 0
QRS duration at diagnosis	Maximal QRS duration on ECG. Select ECG picked for "DateECG", if not on class 1 anti-arrhythmics or amiodarone. If on these medication on that ECG, select another one off medication that is closest from diagnosis if possible. Milliseconds
Terminal Activation Delay at diagnosis	Terminal activation duration of QRS measured from the nadir of the S wave to the end of the QRS, including R', in V1, V2, or V3, in the absence of complete right bundle-branch. Yes = 1, no = 0
Bundle branch block at time of diagnosis	Presence of bundle branch block (on ECG selected for "ECG at diagnosis"). Right bundle branch defined as: 1- QRS duration greater than or equal to 120 ms in adults, greater than 100 ms in children ages 4-16 years and greater than 90 ms in children less than 4 years of age 2- rsr' rsR' or rSR' in leads V1, or V2. The R' or r' deflection is usually wider than the initial R wave. In a minority of patiens, a wide and often notched R wave pattern may be seen in lead V1 and/or V2 3- S wave of greater duration than R wave or greater than 40 ms in leads I and V6 in adults 4- Normal R peak time in leads V5 and V6 but greater than 50 ms in lead V1 Of the above criteria, the first 3 should be present to make the diagnosis. When a pure dominant R wave with or without a notch is present in V1, criterion 4 should be satisfied. Left bundle branch block defined as:

	1-QRS duration greater than or equal to 120 ms in adults greater than 100 ms in children 4-16 years of age and greater than 90 ms in children less than 4 years of age. 2-Broad notched or slurred R wave in leads I, aVL, V5 and V6 and an occasional RS pattern in V5 and V6 attributed to displaced transition of QRS complex. 3-Absent q waves in leads I V5 V6 but in the lead aVL, a narrow q wave may be present in the absence of myocardial pathology 4-R peak time greater than 60 ms in leads V5 and V6 but normal leads V1,V2 and V3 when small initial r waves can be discerned in the above leads No = 0, RBBB = 1, LBBB = 2
Number of leads with T-Wave Inversions in anterior leads	Number of precordial leads with T-wave inversion (V1 through V6). (on ECG selected for "ECG at diagnosis"). T-waves are considered inverted if amplitude ≥ 1 mV (1 mm). Number
Number of leads with T-Wave Inversions in inferior leads	Number of inferior leads with T-wave inversion (II, III, aVF). (on ECG selected for "ECG at diagnosis"). T-waves are considered inverted if amplitude ≥ 1 mV (1 mm). Number
AMBULATORY CARDIAC MONITORING PARAMETERS	
Holter at Diagnosis	Was Holter performed at diagnosis? (prior to one year after dx/or first event) Yes = 1, No = 0
Max PVC count on Holter	Maximum PVC count on a 24 hrs Holter (prior to one year after dx/or first event, Prioritize 1-year time frame before and after dx). Number
NSVT at diagnosis	History of Non sustained VT (NSVT) on any exam at diagnosis (At any time prior to one year after dx/or first event). NSVT is defined as 3 or more consecutive ventricular beats at a rate of >100 beats per minute with duration of less than 30 seconds and without hemodynamic compromise. Yes = 1, No = 0
ECHOCARDIOGRAPHIC PARAMETERS	
Echo at Diagnosis	Transthoracic echocardiogram performed at diagnosis? (prior to one year after dx/or first event). If a patient has more than one exam with the same imaging technique, the exam with the most complete and reliable report that is the closest from the date of diagnosis will be selected for coding. Prioritize 1-year time frame before and after dx Yes = 1, No = 0
Qualitative assessment of RV dilation on Diagnosis Echocardiogram	Qualitative global assessment of RV volume on ECHO based on category. Normal, mild dilatation, moderate dilatation, severe dilatation
Echo parasternal long axis RVOT measurement at diagnosis	Measure of right ventricular outflow tract (RVOT) in parasternal long axis on transthoracic echocardiogram. millimeters
Echo parasternal short axis RVOT	Mesure of RVOT in parasternal short axis on transthoracic echocardiogram.

measurement at diagnosis	millimeters
Echo RVEF	RV ejection fraction (RVEF) as measurement for RV dysfunction on transthoracic echo. %
Echo RVFAC	RV fractional area change on transthoracic echocardiogram %
Echo LVEF	Left ventricle ejection fraction (LVEF) as measurement for LV dysfunction on transthoracic echo. %
MRI PARAMETERS	
MRI at time of diagnosis	Magnetic resonance imaging (MRI) performed at diagnosis? (prior to one year after dx/or first event) If a patient has more than one exam with the same imaging technique, the exam with the most complete and reliable report that is the closest from the date of diagnosis will be selected for coding. Prioritize 1-year time frame before and after dx Yes = 1, No = 0
BSA	Body surface area. (Ideally on MRI report, if not available take one from another test like echo or calculate from the medical chart with Mosteller formula. Use values as close as possible to the date of MRI) m^2
MRI Right ventricular Volume	Right ventricular end-diastolic volume (RVEDV) on MRI. (on MRI chosen for MRI at time of diagnosis). mL
MRI RVEF	RV ejection fraction as measurement for RV dysfunction on MRI. %
MRI LVEF	LV ejection fraction as measurement for LV dysfunction on MRI. %
IMPUTED IMAGING PARAMETERS	
RVEF	Right ventricular ejection fraction. Manual imputation for RVEF: 1-RVEF on CMR is preferred for RVEF assessment 2-For patients with assessment of RV function both with ultrasound and CMR: We will compare the qualitative ultrasound value, establish the median value of MRI RVEF associated with each qualitative category (normal function, mild dysfunction, moderate dysfunction, severe dysfunction) 3-For patient with ultrasound-only assessed RV function, the median value calculated in step 2 will be assigned for the primary analysis 4-A secondary sensitivity analysis will compare this method with the use of RVEF on MRI only with the use of standard multiple imputation based on chained equation to handle missing values 5-For patients with both FAC and RVEF by MRI, a conversion factor will be determined 6-Patients who only have RV function assessment by FAC will be assigned a RVEF with the method described in 5. 7- Patients who only have a qualitative assessment of normal RVEF by MRI, will be assigned the median value of patients with normal MRI RV function (above 45%) Percentage.

LVEF	Left Ventricular ejection fraction. Manual imputation for LVEF as below: 1-LVEF on CMR is preferred for LVEF assessment. 2-If LVEF on CMR is not available, quantitative assessment by cardiac ultrasound will be used **3-** For patients with assessment of LV function both with ultrasound and MRI, we will compare the qualitative ultrasound value, establish the median value of MRI LVEF associated with each qualitative category (normal, mild dysfunction, moderate dysfunction, severe dysfunction) 3-For patient who only have a qualitative ultrasound assessment of LV function, the median value calculated in step 2 will be assigned If the number of patients with both a qualitative echocardiographic assessment of LVEF and quantitative MRI assessment is too low for one category of dysfunction; Normal will be imputed to 65%, mild dysfunction 50%, moderate dysfunction 40% and severe dysfunction 30% %
ICD PARAMETERS	
ICD	ICD implanted at any time Yes = 1, No = 0
Age at ICD implantation	Date of first ICD implantation Days
ICD Monitoring Zone at the time of implant	Cycle length of the Monitor zone at implant milliseconds
ICD Treatment Zone at time of implant	Cycle length of the lowest therapy zone at implant milliseconds
ICD Monitoring Zone at last follow up	Cycle length of the monitor zone at first LTVA or last programing available at follow-up milliseconds
ICD Treatment Zone at last follow up	Cycle length of the lowest therapy zone at first LTVA or last programing available at follow-up milliseconds
OUTCOME PARAMETERS	
Last Clinical Follow up	Age at the time of last clinical follow-up allowing assertion of outcomes. days
Life threatening ventricular arrhythmia (LTVA) after diagnosis	Composite outcome of first life threatening ventricular arrhythmia. Comprised of sustained ventricular tachycardia (VT lasting ≥ 30 secs or with hemodynamic compromise at ≥ 100bpm or terminated by electrical cardioversion), appropriate ICD intervention (ICD shock or antitachycardia overdrive pacing delivered in response to a ventricular tachyarrhythmia according to stored intracardiac ECG data), aborted sudden cardiac arrest (An event as described above, that is reversed, usually by cardiopulmonary resuscitation and/or defibrillation or cardioversion), or sudden cardiac death (Death of cardiac origin that occurred unexpectedly within 1 hour of the onset of new symptoms or a death that was unwitnessed and unexpected). None = 0, Sustained VT = 1, ICD intervention = 2, SCA = 3, SCD = 4

Age at first life threatening VA event	Age of 1st composite outcome of first life threatening ventricular arrhythmia Days
LTVA cycle length	Cycle length of ventricular arrhythmia coded for primary outcome Milliseconds
Severe LTVA after time of diagnosis	VT with CL≤ 240 ms(≥250 bpm), FV, SCD or resuscitated SCD. Defined as above. None = 0, Sustained VT = 1, Appropriate ICD intervention = 2, SCA = 3, SCD = 4
Age at time of first severe LTVA event	Age at 1st Severe VA (VT with CL≤ 240 ms[≥250 bpm] or FV, SCD or resuscitated SCD) Days
Severe LTVA cycle length	Cycle length of the first severe LTVA event Milliseconds
Transplant	Cardiac transplant at follow up Yes = 1, No = 0
Age at time of transplant	Age at time of cardiac transplant during follow up Days
Death	Death during follow up Yes = 1, No = 0
Age at death	Age at the time of death during follow up Days
Cause of death	Categorical cause of death SCD = 1, Heart failure = 2, Arrhythmic and heart failure (e.g. heart failure caused by arrhythmias), 4 = non-cardiac cause of death
VT ablation	Endocardial or epicardial VT ablation performed at any time before last coded event Yes = 1, No = 0
Age at time of VT ablation	Age at the time of first ablation Days

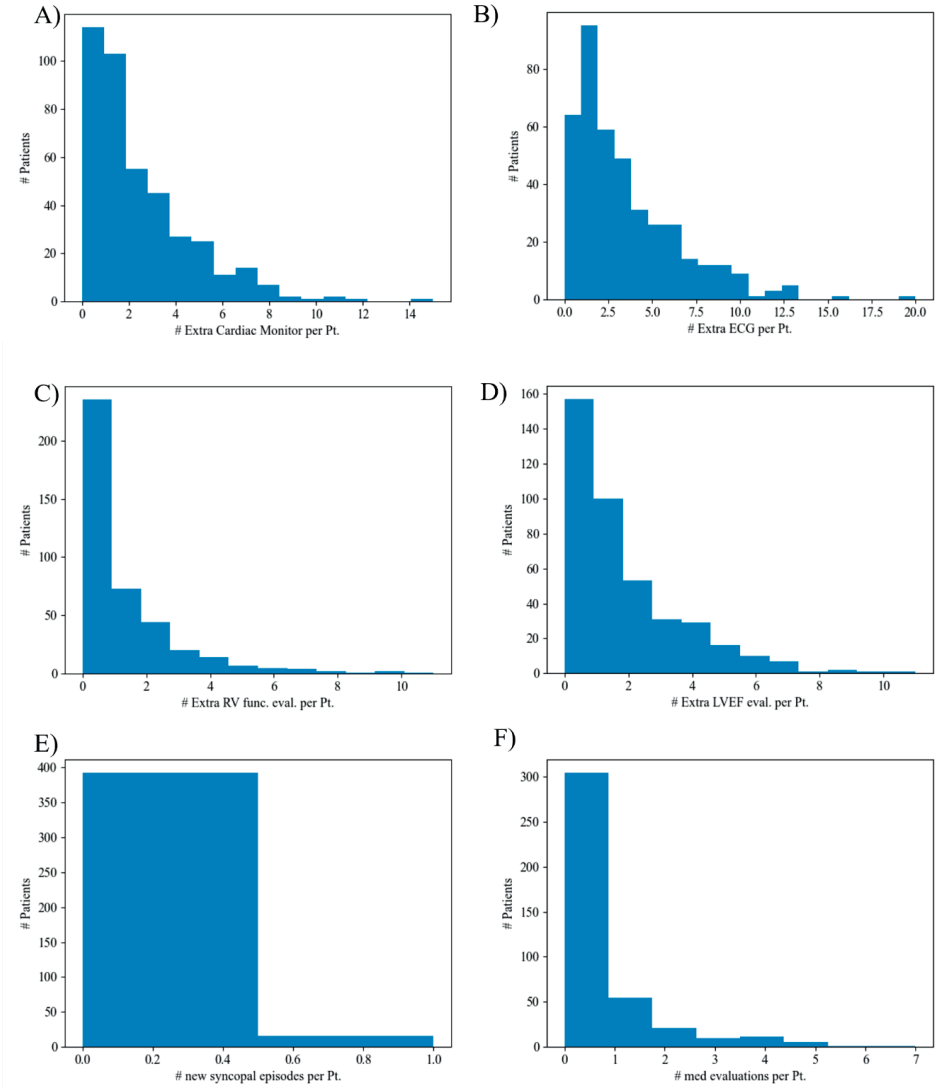
Table II: Comparison of baseline patient characteristics between JHH ARVC registry, Netherlands AVC registry, and the ARVC risk calculator derivation cohort.

Variable	Netherlands AVC Registry (n=146)	JHU ARVC Registry (n=262)	p value	Cadrin-Tourigny et al., 2019 {Cadrin-Tourigny, 2019 #3} (n=528)
Age at Diagnosis (n=408)	42 (±14.5)	34 (±14.7)	<0.001	38.16 ± 15.47
Male Sex (n=408)	63 (43.2%)	101 (38.5%)	0.364	236 (44.7%)
Caucasian Race (n=407)	144 (98.6%)	253 (96.9%)	0.289	485 (91.9%)
Pathogenic Variant (n=405)	114 (78.1%)	184 (71.0%)	0.123	340 (64.4%)
PKP2	87 (59.6%)	110 (42.5%)	<0.001	258 (48.9%)
DSP	1 (0.7%)	12 (4.6%)	0.03	23 (4.4%)
DSG2	1 (0.7%)	10 (3.9%)	0.059	17 (3.2%)
PLN	23 (15.8%)	4 (1.5%)	<0.001	26 (4.9%)
Other	0 (0.0%)	13 (5.0%)	0.006	10 (1.9%)
Symptoms (n=361)	95 (65.1%)	137 (63.7%)	0.793	307 (58.1%)
History of Cardiac Syncope (n=408)	32 (21.9%)	45 (17.2%)	0.241	107 (20.3%)
Anterior T-wave inversions (n=398)	3 [1.0; 4.0]	3 [2.0; 4.0]	0.152	Not Reported
Inferior T-wave inversions (n=387)	0 [0.0; 1.0]	0 [0.0; 1.0]	0.98	Not Reported
Total T-wave inversions (ant.+inf.) (n=387)	3 [1.8; 5.0]	3 [2.0; 5.0]	0.228	Not Reported
24 hr. PVC count (n=343)	1147 [517.0; 3398.5]	1234 [313.0; 4227.0]	0.805	1007 [278; 3731]
Presence of NSVT (n=370)	71 (51.8%)	124 (53.2%)	0.795	231 (43.8%)
RVEF (%) (n=348)	45 (±8.0)	43 (±11.2)	0.039	43.80 ± 10.40
LVEF (%) (n=355)	57 (±7.3)	58 (±8.4)	0.074	57.66 ± 8.42
ICD (n=407)	90 (61.6%)	187 (71.6%)	0.038	218 (41.3)
Anti-arrhythmic Prescription (n=391)	28 (19.2%)	31 (12.7%)	0.081	82 (15.5)
Beta-Blocker Prescription (n=392)	49 (33.6%)	104 (42.3%)	0.087	200 (37.9)
ARVC calculator 5-year VA risk (n=408)	25 (±20%)	31 (±25%)	0.012	Not Reported

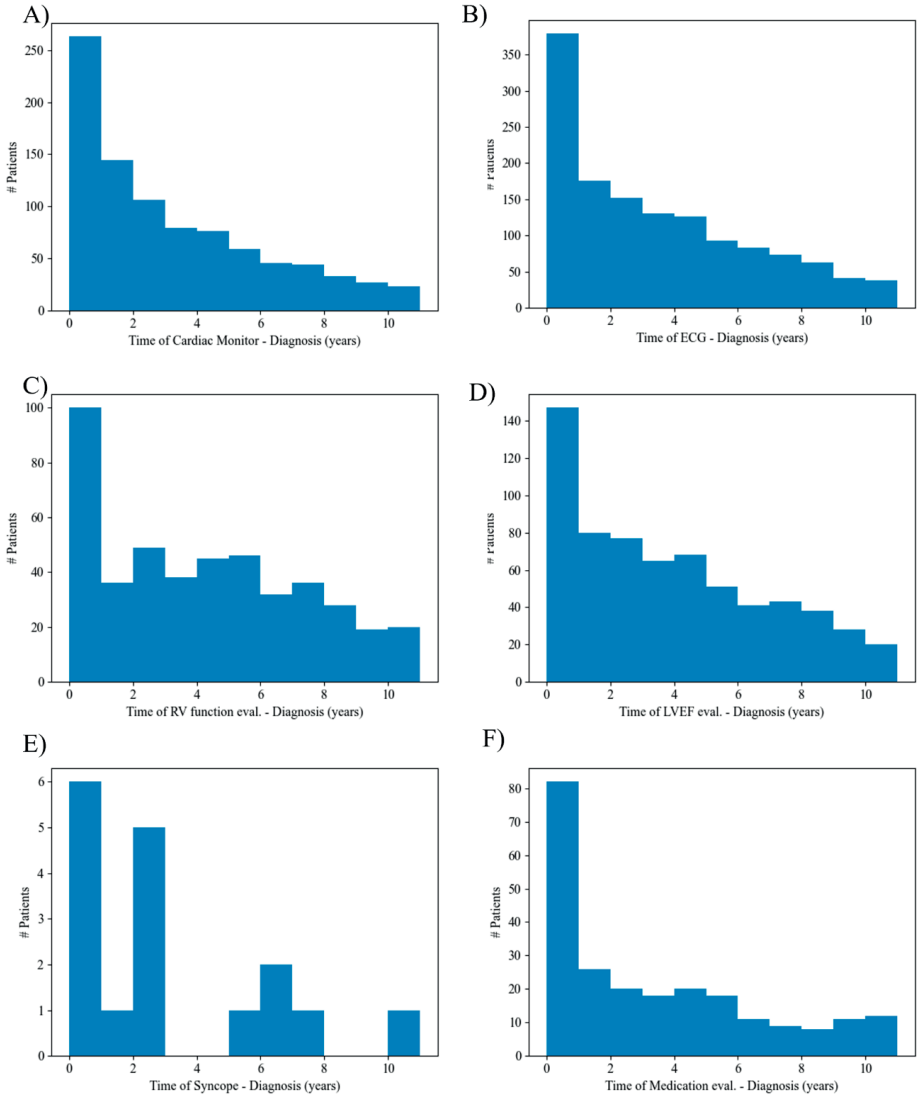
Supplemental Table III: Empirically determined γ and τ parameters used for modeling the discrepancy between the ARVC risk calculator (updated) predictions and observed risk, both for the total cohort, as well as for low (0-10% baseline risk), intermediate (10-25% baseline risk), and high (>25% baseline risk) risk subgroups.

Cohort	γ	τ
Total Cohort	6.2	1.3
Low Risk	2.4	153
Intermediate Risk	9.1	2
High Risk	12.7	0.3

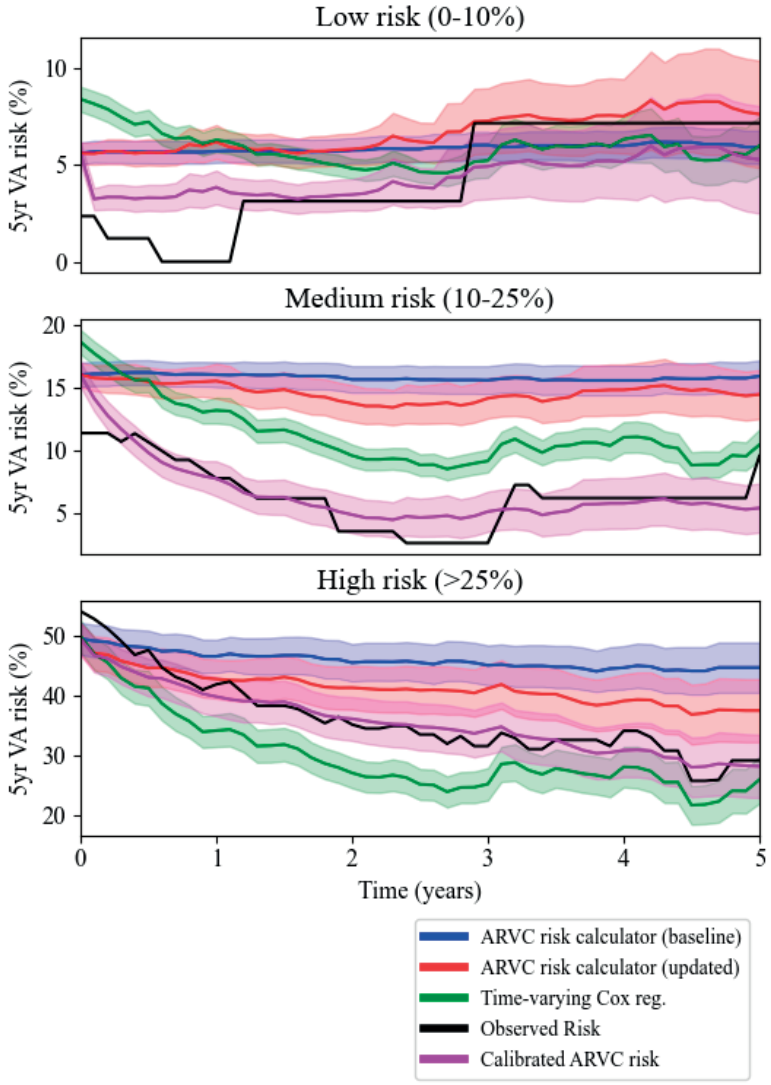
Supplementary Figure I: Histograms demonstrating the distributions of additional A) cardiac monitors, B) ECGs, C) follow up evaluations of RV function, D) follow up evaluations of LVEF, E) new syncopal events, and F) repeat medication evaluations per patient.



Supplementary Figure II: Histograms demonstrating the distributions of timing relative to ARVC diagnosis (out to for additional A) cardiac monitors, B) ECGs, C) follow up evaluations of RV function, D) follow up evaluations of LVEF, E) new syncopal events, and F) repeat medication evaluations per patient.



Supplementary Figure III: Calibration curves for low (0-10% baseline predicted risk), intermediate (10-25% baseline predicted risk) and high (>25% baseline predicted risk) risk groups.



CHAPTER 9

LONG-TERM ARRHYTHMIC FOLLOW-UP AND PERFORMANCE OF MODERN RISK STRATIFICATION TOOLS IN LARGE COHORT OF PATIENTS WITH DESMOPLAKIN ARRHYTHMOGENIC CARDIOMYOPATHY

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Submitted

ABSTRACT

Background: Data describing arrhythmic outcomes of patients harboring a likely pathogenic/pathogenic (LP/P) desmoplakin (*DSP*) variant and fulfilling a definite diagnosis of arrhythmogenic cardiomyopathy (ACM) is scarce. There has been limited assessment of clinical and demographic variables associated with ventricular arrhythmias (VAs) and the performance of a current risk stratification algorithm (ARVC risk calculator) in this population is uncertain.

Aims: To characterize arrhythmic outcomes and to test the ARVC risk calculator performance in patients with DSP-associated ACM fulfilling 2010 Task Force Criteria over long-term follow-up.

Methods: Patients with a definite ACM diagnosis and harboring a LP/P *DSP* variant were enrolled from eighteen ACM/cardiomyopathy registries in North America, Europe, and Australia. VA events, defined as a composite of sustained ventricular tachycardia (VT), appropriate implantable cardioverter defibrillator therapies, and ventricular fibrillation/sudden cardiac death events in follow up, were reported as the primary outcome. The performance of the ARVC risk score for VA prediction in eligible patients was tested, reporting c-statistic and calibration plots.

Results: Among 252 DSP-ACM patients (39.6±16.9 years old, 35.3% male) enrolled in the study, 94 (37.3%) experienced a VA event over a median follow-up of 44.5 [19.6–78.3] months. History of previous non-sustained VT (aHR 2.249; p=0.001) showed the strongest association with the study outcome, while neither age (p=0.723) nor male sex (p=0.200) were associated. In the 204 patients with no VA at diagnosis and thus eligible for risk stratification with the ARVC risk calculator, overall performance of the algorithm was poor (c-statistic 0.604 [0.594–0.614]). Performance was reasonable in the 58 (28.4%) of DSP-ACM patients without LV involvement (c-statistic 0.691 [0.678–0.704]) but very poor the larger group (N=146, 71.6%) of DSP-ACM patients with LV disease (c-statistic 0.561 [0.558–0.564]).

Conclusion: Patients with DSP-ACM are at high risk for VAs, with previous non-sustained VT representing the strongest risk factor. The current ARVC risk calculator showed poor performance in DSP-ACM patients with LV involvement. A gene-specific risk calculator may provide better VA risk stratification for these patients.

INTRODUCTION

Arrhythmogenic cardiomyopathy (ACM) is a heterogeneous genetic disease characterized by fibro-fatty infiltration of the myocardium and the development of potentially lethal ventricular arrhythmias (VA)[1]. In light of this elevated risk for sudden cardiac death (SCD), the usual next step in clinical management following a patient's ACM diagnosis is individualized assessment of arrhythmic risk and a decision regarding the placement of an implantable cardioverter defibrillator (ICD)[2,3]. However, while current guidelines agree upon the clear benefits of offering ICD for secondary prevention of sustained VA, the indications for primary prevention ICD use in patients with ACM have historically been less clear.

In 2019, a novel risk stratification tool for aiding in ICD decision making for patients with a definite diagnosis of ACM but no previous sustained VA events (the ARVC risk calculator, available at www.arvcrisk.com) was proposed by a multinational collaboration[4]. Since then, the ARVC risk calculator has been found reliable in multiple external validation cohorts[5–11], with performance superior to standard stratification algorithms in protecting patients from VAs with an overall lower number of amount of ICDs placed per treated event[12]. This risk stratification tool, however, was derived from an ACM patient cohort primarily composed of classic plakophilin-2 (*PKP2*) variant carriers and of gene-elusive ACM patients[4]. Not surprisingly, studies have suggested sub-optimal performance of the ARVC risk calculator in left dominant forms of ACM, although the relatively low patient sample size and event rate of those studies precluded definite conclusions[5,7,10].

Pathogenic desmoplakin (*DSP*) variants are associated with an ACM phenotype in which the left ventricle (LV) is often extensively affected even at early stages of disease. LV dominant,

biventricular, and right ventricular (RV) dominant ACM-DSP have all been described, with LV involvement resulting the most common phenotype[13,14]. However, long-term outcome characterization of DSP-ACM phenotypes has been limited to small sample sized cohorts[13–15], and around half of the *DSP* patients in these cohorts did not fulfill 2010 Task Force Criteria (TFC) for definite diagnosis of ACM. Furthermore, even when a definite diagnosis of ACM is reached, it remains unclear whether current risk stratification strategies can be applied effectively to this specific sub-population of ACM patients[5,7,10]. This study therefore aims to a) assess long-term outcomes in a large, multinational cohort of patients fulfilling definite ACM diagnosis associated with a *DSP* variant (DSP-ACM) and b) to assess the performance of the ARVC risk calculator for predicting VA events in this important subgroup of ACM patients.

METHODS

Study Cohort

The current study was planned as a cohort study. Patients were extracted from the ACM and genetic cardiomyopathy registries of 18 academic institutions from 8 different countries (United States of America, United Kingdom, France, Italy, the Netherlands, Canada, Australia, and Switzerland). Each registry is in itself a longitudinal cohort study.

From each registry, patients were included in the present study if they:

- Harbored a pathogenic (P) or likely pathogenic (LP) genetic variant in *DSP* per the American College of Medical Genetics and Genomics (ACMG) criteria[16];
- Fulfilled a definite diagnosis of ACM in accordance to the 2010 Task Force Criteria (TFC)[1];
- Had at least one cardiac imaging test available (Cardiac magnetic resonance (CMR) or echocardiography) at the time of TFC fulfillment;
- Had of at least one day of follow up available for outcome ascertainment.

Ethical review board approval and written patient consent were obtained, in accordance with local regulations. The study was performed in accordance with the Declaration of Helsinki.

Data Collection

Data were collected independently at each site, according to a pre-determined data collection spreadsheet (as reported in Section A of the Supplementary Materials). Available demographics, patient medical history, genetic test results, baseline cardiac instrumental exams (12-lead electrocardiogram (ECG), echocardiography, CMR, 24-h Holter-ECG monitor) were retrieved for each patient. All *DSP* genetic variants initially considered P or LP locally underwent expert review by core lab from specialists in cardiac genetics (B.M., C.J), in accordance with the American College of Medical Genetics and Genomics guidelines[17]. A list of all genetic variants included in the study has been reported in **TableS1**. Non-sustained ventricular tachycardia (NSVT) has been defined as a three or more subsequent premature ventricular complexes at a frequency >120 bpm. Heart failure (HF) episodes were defined as a clinical presentation consistent with acute or decompensated HF requiring hospitalization, emergency department access, or medical therapy changes. The presence of LV involvement was defined as the presence of late gadolinium enhancement in the LV on CMR and/or the presence of an LV ejection fraction (LVEF) ≤45% on any cardiac imaging test.

Study outcomes

Consistent with the published ARVC risk calculator, the primary outcome was first sustained VA following confirmed ACM diagnosis[10,11,18]. Sustained VA was defined as a composite of the occurrence of sudden cardiac arrest (SCA), spontaneous sustained ventricular tachycardia (VT) lasting ≥ 30s with a frequency of at least 100 bpm or with hemodynamic compromise, ventricular fibrillation/flutter (VF), or appropriate ICD intervention[4–11,18]. The primary prevention cohort was composed of those patients with no history of sustained VA at the time of TFC fulfillment. Secondary outcomes included episodes of decompensated heart failure, heart transplantation, as well as cardiovascular and all-cause mortality.

Statistical Analysis

Analyses were performed in PyCharm software version 2021.2.2 (JetBrains Inc., Boston, MA, USA) and the open-source Pandas, Lifelines, and Statsmodels statistical code libraries. Categorical

variables were summarized as frequencies (%) and compared using proportional *z*-tests. Continuous variables were presented as mean ± standard deviation or median [interquartile range (IQR)], and compared using independent sample Students *t*-tests or the Mann-Whitney *U*-tests, as appropriate. Follow-up duration was calculated from the date of definite ACM diagnosis to the date of first sustained VA event or censoring, which was defined as death from any other cause, heart transplantation, or the most recent follow-up visit at which the endpoints could be ascertained. The overall probability of survival free from sustained VA was estimated using the Kaplan-Meier method. Rates of incident VA are reported as averages over the 5-year period following initial diagnosis, both within the overall cohort and stratified by both (1) presence/absence of sustained VA prior to ACM diagnosis (primary vs secondary prevention cohort), and (2) presence/absence of LV involvement. Log-rank (LR) testing was used to assess differences in VA event rates between subgroups. Associations between individual risk factors and sustained VA events were assessed using Cox proportional hazards regression models; those risk factors for whom p-value was < 0.1 were included in a subsequent multivariable Cox proportional hazard regression model.

ARVC risk calculator

As previously described[4], the ARVC risk calculator offers individualized assessment of risk for incident sustained VA events based on 7 clinical risk factors for VA (age (years); sex; 24-hour burden of premature ventricular contractions (PVCs); right ventricular ejection fraction (RVEF, %); the number of T wave inversion in anterior and inferior leads on 12-lead electrocardiogram; history of NSVT; recent (within the previous 6 months) cardiac syncope), and is calculated according to equation 1.

(1) $5yr\ VA\ Risk = 1 - 0.8396^{\exp(PI)}$

Here, the prognostic index PI) is calculated according to equation 2.

(2) $PI = 0.488 * Male\ Sex - 0.022 * Age + 0.657 * cardiac\ syncope + 0.811 * NSVT + 0.170 * \ln(PVC\ burden) + 0.113 * TWI - 0.025 * RVEF$

Missingness in data for the predictors included in the ARVC risk calculator were assumed to be at random and imputed using multiple imputation with chained equations[19]. The final imputation model included all predictors from the ARVC risk calculator along with VA incidence and a cumulative baseline hazard estimation. A total of 20 imputed datasets were generated using 20 iterations each, and the final estimates were combined using Rubin's rule[20]. The ARVC risk calculator's ability to discriminate risk for sustained VA was assessed using concordance based c-statistics, with 95% confidence intervals generated from 5-fold cross validation. Calibration was assessed using calibration plots.

RESULTS

Patient Cohort

A total of 252 patients were included in the study. The mean age at TFC fulfillment was 39.6±16.9 years and 89 (35.3%) patients were male. Probands made up 59.5% of the cohort. At ACM diagnosis, the vast majority (84.9%) of patients had >500 PVCs/24h, with the median 24-h PVC burden of 2000 [650–5000] PVCs. Mean LVEF and RVEF of the study cohort were mildly reduced, 45.0±13.3% and 46.4±11.2%, respectively. LV involvement was observed in 179 (71.0%) patients. **Table1** summarizes the baseline characteristics of the cohort. At the time of ACM diagnosis, 204 (81.0%) patients had no history of sustained VA events (primary prevention cohort), while 48 (19.0%) had experienced at least one previous VA event. The baseline characteristics of the primary prevention patient cohort are reported in **Table2.**

Table1

Overall Cohort Characteristics (n=252)	
Age at TFC fullfillment , mean±s.d	39.6±16.9
Male sex, n (%)	89 (35.3)
Probands, n (%)	150 (59.5)
TFC **f**ulfillment	252 (100)
Class I – Structural (Assessed n=252)	
Major, n (%)	71 (28.2)
Minor, n (%)	24 (9.5)
Class II – Tissue Characterization (Assessed n=37)	
Major, n (%)	3 (8.1)
Minor, n (%)	9 (24.3)

Class III – Depolarization (Assessed n=252)	
Major, n (%)	16 (6.3)
Minor, n (%)	75 (29.8)
Class IV – Repolarization (Assessed n=252)	
Major, n (%)	93 (36.9)
Minor, n (%)	94 (37.3)
Class V – Arrythmias (Assessed n=248)	
Major, n (%)	34 (13.7)
Minor, n (%)	182 (73.4)
Class VI – Family History (Assessed n=252)	
Major, n (%)	252 (100)
Sustained VA at/prior to TFC fulfillment, n (%)	48 (19.0)
ECG	
Overall n of TWI, median [IQR]	3 [1–4]
TWI in ≥ 3 precordial leads, n (%)	124 (49.2)
TWI in ≥ 2 inferior leads, n (%)	45 (17.9)
24-h PVC burden, median [IQR]	2000 [650–5000]
24-h PVC burden ≥ 500, n (%)	214 (84.9)
LVEF at TFC fulfillment , mean±s.d	45.0±13.3
RVEF at TFC fulfillment, mean±s.d	46.4±11.2
LGE at TFC fulfillment, mean±s.d (Assessed n=199)	131 (78.9)
LV disease involvement, mean±s.d	179 (71.0)
ICD at TFC fulfillment, mean±s.d	118 (46.8)

Table2

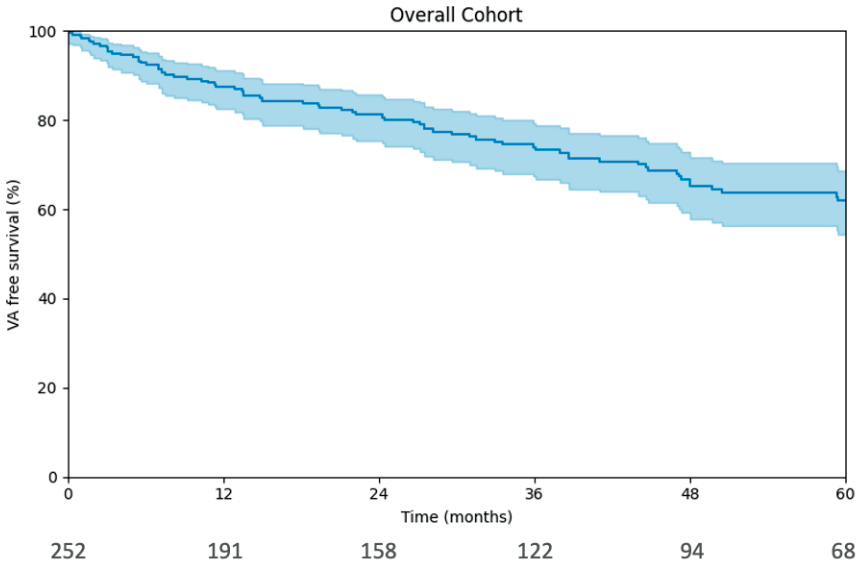
Primary Prevention Baseline Characteristics (n=204)	
Age at TFC fulfillment, mean±s.d.	39.1±17.4
Male sex, n (%)	65 (31.9)
ECG	
Overall n of TWI, median [IQR]	3 [1–4]
TWI in ≥ 3 precordial leads, n (%)	95 (46.6)
TWI in ≥ 2 precordial leads, n (%)	36 (17.6)
History of NSVT, n (%)	59 (28.9)
History of Cardiac Syncope, n (%)	22 (10.8)
24-h PVC Burden	1920 [612–5000]
RVEF at TFC fulfillment, mean±s.d	46.6±10.5
LVEF at TFC fulfillment, mean±s.d	45.8±13.4
LGE at TFC fulfillment, n (%) (n=166)	111 (66.9)
LV disease involvement, n (%)	146 (71.6)
ARVC risk overall, median [IQR]	15.4 [8.3–25.0]
ARVC risk no LV involvement, median [IQR]	11.1 [6.3–20.3]
ARVC risk LV involvement, median [IQR]	16.4 [8.9–26.5]
ICD at TFC fulfillment, n (%)	81 (39.7)

Outcomes

Table3 summarizes study outcomes for the overall and primary prevention cohorts stratified by LV involvement. Over a median follow up of 44.5 [19.6–78.3] months, 94 (37.3%) patients experienced a sustained VA event. **Figure 1** reports the KM curve for the entire cohort. Patients with a prior VA

event at the time of ACM diagnosis, experienced a higher arrhythmic event rate compared to the primary prevention cohort (Log Rank p-value=0.034).

Figure1



Overall arrhythmic sustained VA in the overall cohort

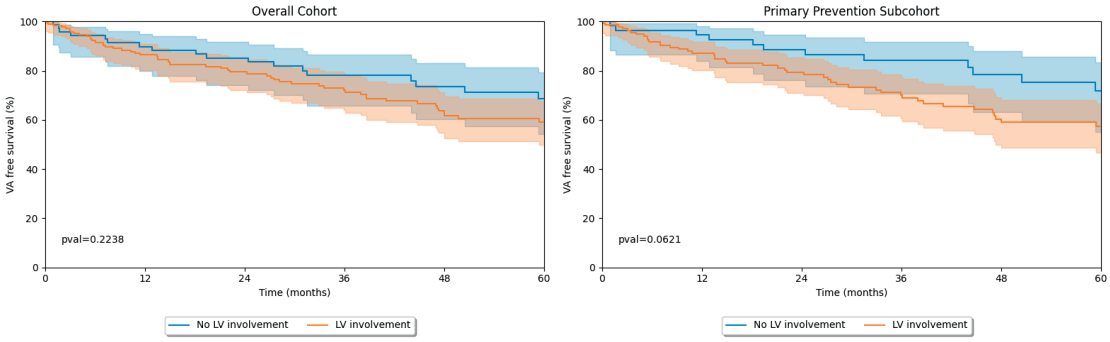
Table3

Follow Up Data						
	Overall Cohort			**Primary Prevention Cohort**		
	Overall (n=252)	LV involvement (n=179)	No LV Involvement (n=73)	Overall (n=204)	LV involvement (n=145)	No LV Involvement (n=59)
Length of fu (months) median [IQR]	44.5 [19.6–78.3]	43.2 [18.7–74.8]	50.7 [25.0–120.0]	44.5 [20.1–78.3]	42.2 [19.0–73.5]	53.4 [26.9–120.0]
VA events, n (%)	94 (37.3)	68 (38.0)	26 (35.6)	67 (32.8)	51 (35.2)	16 (27.1)
Sustained VT, n (%)	30 (11.9)	21 (11.7)	9 (12.3)	26 (12.7)	19 (13.1)	7 (11.9)
ICD shocks, n (%)	57 (22.6)	42 (23.5)	15 (20.5)	36 (17.6)	28 (19.3)	8 (13.6)
VF/SCA, n (%)	7 (2.8)	5 (2.8)	2 (2.7)	5 (2.5)	4 (2.8)	1 (1.7)
HF episodes, n (%)	47 (18.7)	37 (20.7)	10 (13.7)	37 (18.1)	30 (20.6)	7 (12.1)
Transplant, n (%)	22 (8.7)	21 (11.7)	1 (1.4)	18 (8.8)	17 (11.6)	1 (1.7)
Death, n (%)	7 (2.8)	6 (3.4)	1 (1.4)	6 (2.9)	5 (3.4)	1 (1.7)
ICD at last fu, n (%)	175 (69.4)	126 (70.4)	49 (67.1)	133 (65.2)	97 (66.4)	36 (62.1)

Overall, no differences in arrhythmia occurrence were observed between patients with and without an LV involvement (**Figure2–Panel A**; LR p-value=0.224), although when only primary prevention patients were considered, a trend towards significance was observed (log-rank p=0.062, **Figure 2–Panel B**). As shown in **Table 3**, during follow-up, 47 (18.6%) patients experienced congestive heart

failure episodes, with 22 (8.7%) patients undergoing heart transplantation. Overall patient mortality at last follow-up was 2.8%. At last available follow-up, 175 (69.4%) patients were implanted with an ICD. Competing-risk sensitivity analysis was performed for non-arrhythmic death and transplant, but did not impact the results. **TableS2** reports association between arrhythmic events during follow-up and baseline clinical characteristics of the overall cohort. In Univariate analysis, PVC burden and an history of non-sustained VT episodes were positively associated with arrhythmic events (HR 1.189 [1.034–1.368], p=0.015;aHR 2.629 [1.655–4.176], p <0.001), while a negative association with RVEF% was observed (HR 0.978 [0.960–0.998], p=0.027). At multivariate analysis, non-sustained VT episodes remained strongly associated with arrhythmic outcomes (aHR 2.249 [1.374–3.682], p=0.001) **Table S3** and S4 shows influence of each component of LV involvement (LVEF, LGE). As can be appreciated, LVEF but not LV LGE were associated with survival free from VA. In multivariate analysis, lower LVEF (aHR 0.977 [0.956-0.998]; p= 0.028) and history of ventricular arrhythmias (aHR 2.236 [1.364-3.663]; p=0.001) were associated with VA in follow-up.

Figure2



Sustained VA rate by presence of LV involvement in the overall cohort (Left Panel) and in the primary prevention cohort (Right Panel)

Performance of the ARVC risk calculator

Performance of the ARVC risk calculator was tested in the primary prevention patient cohort (n=204). In the primary prevention cohort, the overall median ARVC risk calculator predicted risk of VA at

5-years was 15.4% [8.3–25.0], and was significantly higher in patients with LV involvement than in those without (16.4% [8.9–26.5] vs 11.1% [6.3–20.3], p=0.012).

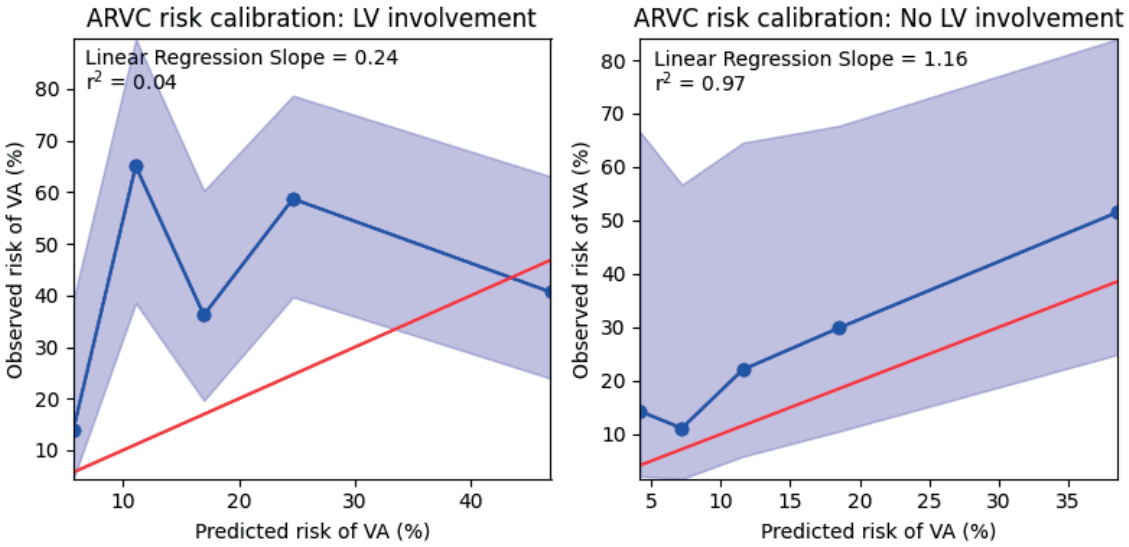
Table3

Follow Up Data						
	Overall Cohort			Primary Prevention Cohort		
	Overall (n=252)	LV involvement (n=179)	No LV Involvement (n=73)	Overall (n=204)	LV involvement (n=145)	No LV Involvement (n=59)
Length of fu (months) median [IQR]	44.5 [19.6–78.3]	43.2 [18.7–74.8]	50.7 [25.0–120.0]	44.5 [20.1–78.3]	42.2 [19.0–73.5]	53.4 [26.9–120.0]
VA events, n (%)	94 (37.3)	68 (38.0)	26 (35.6)	67 (32.8)	51 (35.2)	16 (27.1)
Sustained VT, n (%)	30 (11.9)	21 (11.7)	9 (12.3)	26 (12.7)	19 (13.1)	7 (11.9)
ICD shocks, n (%)	57 (22.6)	42 (23.5)	15 (20.5)	36 (17.6)	28 (19.3)	8 (13.6)
VF/SCA, n (%)	7 (2.8)	5 (2.8)	2 (2.7)	5 (2.5)	4 (2.8)	1 (1.7)
HF episodes, n (%)	47 (18.7)	37 (20.7)	10 (13.7)	37 (18.1)	30 (20.6)	7 (12.1)
Transplant, n (%)	22 (8.7)	21 (11.7)	1 (1.4)	18 (8.8)	17 (11.6)	1 (1.7)
Death, n (%)	7 (2.8)	6 (3.4)	1 (1.4)	6 (2.9)	5 (3.4)	1 (1.7)
ICD at last fu, n (%)	175 (69.4)	126 (70.4)	49 (67.1)	133 (65.2)	97 (66.4)	36 (62.1)

Over a 5-year follow up period, 57 (27.9%) primary prevention patients experienced a sustained VA event. **Table4** reports association between risk factors included in the ARVC risk calculator and these arrhythmic outcomes. In multivariable cox regression, a previous episode of NSVT remained significantly associated with a higher risk of sustained VA events during 5-year follow up (aHR 1.815 [1.035–3.184], p=0.038), while male sex was associated with trend tower a lower risk (aHR 0.539 [0.279–1.043], p=0.067). No association between age and arrhythmic risk was observed, while presence of LV involvement showed only a trend towards significance in the univariable analysis.

Discrimination of sustained VA risk by the ARVC risk calculator was poor within the primary prevention cohort (c-statistic 0.604 [0.594–0.614]) (**Figure S1**). Both discriminative performance and calibration of the ARVC risk calculator was very poor in patients with LV involvement (c-statistic 0.561 [0.558–0.564]), but better in those that without (0.691 [0.678–0.704]) (**Figure3**– Panel A&B). Per-risk bracket performance of the ARVC risk calculator in arrhythmic risk stratification for the primary prevention DSP-ACM cohort has been graphically displayed in **Figure S2** (overall) and **Figure S3&S4** (stratification by LV-involvement).

Figure3



Calibration plot for the performance of the ARVC Risk Calculator in the primary prevention cohort if LV involvement is present (left panel) or absent (right panel)

DISCUSSION

This multi-national study enrolled the largest cohort of patients with both a definite diagnosis of ACM and a LP/P *DSP* variant that has been published to date. We described the long-term clinical outcomes of this cohort and reported the performance of the ARVC risk calculator for VA event prediction in this important subgroup of ACM patients.

The main findings of this study are summarized as follows:

- During a median follow up of almost 4 years, a high rate of VA events (overall 37.3%; annualized over 5-yr 7.6% [6.2–9.2%]) was observed among patients with a definite diagnosis of ACM and a P/LP *DSP* variant;
- The observed VA rate was equally high (overall 32.8%; annualized over 5-yr 7.7% [6.1-9.4%]) among those patients without previous VA event at the time of ACM diagnosis (“primary prevention patients”);

- In multivariable analysis, a history of NSVT was associated with an increased risk of VA events in primary prevention patients; interestingly, there was no difference in risk between male and female patients.
- The current ARVC risk calculator showed an overall poor performance in VA risk discrimination in this patient population (Overall C statistic 0.604 [0.594–0.614]);
- When stratifying for patients with and without an LV disease involvement, the ARVC risk score performance was reasonable in DSP-ACM patients without LV involvement (C statistic 0.691 [0.678–0.704]), but very poor in patients with LV involvement (C statistic 0.561 [0.558–0.564])).

DSP & ACM

Patients with LP/P *DSP* variants can develop a wide spectrum of disease phenotypes, ranging from dilated cardiomyopathy to left, right, or biventricular ACM[13,14,21]. When these patients reach a definite diagnosis of ACM, there is no clear consensus on the relative arrhythmic risk compared to the other ACM phenotypes (i.e. *PKP2*-ACM,, gene-elusive ACM). In comparison to classical ACM phenotypes, higher, similar, or lower VA rates have been reported for patients with *DSP* variants[10,13–15,21,22]. Furthermore, clinical and demographic predictors of risk of VA, particularly in patients with no sustained VA history at diagnosis is largely unknown. Prior studies have been hampered by small patient sample sizes, and a definitive answer regarding arrhythmic risk in these patients remains elusive. The main aim of this study was therefore to address ventricular arrhythmia outcomes of DSP-ACM in a large patient population and to assess arrhythmic predictors in these patients.

A high rate of VA events was observed in our study cohort, with more than a third of DSP-ACM patients experiencing sustained ventricular arrhythmias during the median 4-year follow up. A previous VA event is known to be strongly associated with additional VA events during follow-up in

patients with ACM[23,24], so the high event rate observed in patients with a previous history of VAs is not surprising. In our cohort, however, VA events were frequent even among those "primary prevention" patients without a history of sustained VA events at the time of ARVC diagnosis (annual event rate 7.7% [6.1-9.4%]). No direct outcome comparisons between different genotypes were performed in this study, but similar primary prevention ACM cohorts primarily comprised of gene elusive and PKP2 patients available in literature reported significantly lower VA rates (Reported annualized VA rates 2.6-5.6%[4,10,11]). These findings strongly point towards DSP-associated ACM being a particularly high arrhythmic risk phenotype.

Arrhythmic Risk Stratification in DSP-associated ACM

Given the high rate of sustained VA events observed in our study, an appropriate arrhythmic risk stratification strategy for patients with DSP-associated ACM is imperative. However, most of the known arrhythmic risk predictors, as well as the entirety of current ACM risk stratification strategies, are based upon data derived from predominantly right-sided ACM cohorts comprised mostly of patients with *PKP2* variants and/or gene-elusive patients[2–4]. Thus, whether classic ACM arrhythmic risk factors remain important within DSP-ACM was previously unknown. In our study, some risk factors for sustained VA within DSP-ACM were similar to those of classical right-sided ACM phenotypes. A high burden of PVC, the presence of NSVT, and a lower RVEF were associated with an increased risk for sustained VA events. At multivariate analysis, the presence of NSVT remained strongly associated with arrhythmic outcomes. To the contrary, other arrhythmic risk factors for classical right sided ACM forms, such as younger age, were not identified as risk factors in this population. Of further particular interest, male patients did not have an increased risk of VA in DSP-ACM. This is in stark contrast to what has been commonly observed in most classical ACM phenotypes and in some previously reported *DSP* cohorts[10,18,21,25]. Furthermore, among primary prevention patients, a trend towards a higher arrhythmic risk for female patients was observed.

These findings are unexpected and represent an important clinical message. While therapeutic intervention in ACM patients has historically been more aggressive in male patients due to their increased arrhythmic risk, this approach may not be appropriate within DSP-ACM. Based on the results of our study, female patients with a *DSP*-associated ARVC should be considered at similar, if not higher, arrhythmic risk than their male counterparts. Further work will be needed to understand why female sex is associated with worse phenotypes in *DSP* cardiomyopathy than in classic ACM.

The ARVC risk calculator has previously been demonstrated to be effective and reliable in discriminating the risk of sustained VA events in primary prevention patients with ACM[10,11]. Comparisons between the available risk stratification algorithms (i.e. TFC 2015[3], HRS 2019[2]) have been performed in multiple independent studies[12,26]. These studies favored the ARVC risk calculator, which achieved greater arrhythmic protection despite a lower total number of implanted ICDs. However, the possibility of underperformance by the ARVC risk calculator in patients with extensive or exclusive LV involvement[5,7], as well as its potential inadequacy for use in specific genotypes, has recently been postulated[10]. In our large cohort of DSP-ACM patients, the ARVC risk calculator's performance was poor overall (C statistic 0.604 [0.594–0.614]). Consistent with previous small reports from *Casella* et al and *Aquaro* et al[5,7], the ARVC risk calculator's discrimination was worst in patients with LV involvement. In contrast, better discrimination was observed in patients without LV involvement (c-statistic 0.691 [0.678–0.704]), although with a trend toward underpredicting likelihood of sustained VA events (**Figure4** – Panel B). These findings partially contradict the report from *Protonotarios* et al, where the ARVC risk score was found to overpredict the arrhythmic risk in patients with DSP-ACM. The observed differences can be explained by the predominance of patients being enrolled from cardiomyopathy centers, resulting in a cohort with lower arrhythmic risk/higher heart failure risk. In our assessment we included a large number of patients from both cardiomyopathy and arrhythmia clinics, in order to capture the whole clinical

spectrum of DSP-ACM patients and minimize center-specific individualities and differences. Finally, in contrast to studies of right sided ARVC phenotypes, the ARVC risk calculator did not perform better than current TFC and clinical consensus guidelines in DSP-ACM, as Figure S2-S4 clearly show. Regardless of the potential threshold of predicted ARVC 5-yr risk, this tool either led to a lower protection rate from arrhythmias that TFC and clinical consensus guidelines or to a similar protection rate but with a way higher number of ICD in place needed to prevent an arrhythmic event (a lower "net benefit" ratio).

Future Perspectives

This study clearly demonstrated that patients with DSP-ACM are at high arrhythmic risk and advocates active arrhythmic surveillance and an aggressive ICD implantation strategy for these patients. The best modality to perform risk stratification assessment in this population, however, remains unclear.

From our data, it seems reasonable to recommend ICD implantation for patients with DSP-ACM and episodes of NSVT, given the strong association of NSVT with complex VA events during follow up. Additionally, patients harboring a *DSP* variant have also been reported as frequently fulfilling DCM criteria[13]. In these patients, considering *DSP* variants as high-risk genetic variants as per the recently released 2022 ESC guidelines for the management of patients with VA (as with variants in phospolamban (*PLN*), filamin C (*FLNC*), and *RBM20)*, seems appropriate[27]. Finally, a genotype tailored risk stratification strategy for ACM has recently been advocated[10,28–30]. A similar approach has been implemented in other genetically-based cardiomyopathies. For example, *Verstraelen* et al. showed that a phospholamban (*PLN*)-tailored risk stratification algorithm was more effective than other available risk stratification scores (i.e. dilated cardiomyopathy guidelines or ARVC risk calculator) for patients with PLN cardiomyopathy [31]. Although their assessment had some significant limitations (they did not assess the performance of the ARVC risk score in *PLN* patients meeting

TFC), the results of this study suggest that such genotype first strategies may be reasonable in other ACM genotypes. It is likely patients with DSP-ACM may benefit from the development of DSP-specific risk stratification tools for the prediction of arrhythmic events.

Limitations

Some limitations of the current cohort study should be highlighted. This was a retrospective cohort study, potentially prone to all biases associated with retrospective studies. To reduce those biases (and in particular selection bias), patients from both arrhythmia clinics and cardiomyopathy/heart failure clinics across the world were enrolled. Additionally, it is well known that physical exercise represents an important determinant of VA events in patients with classical right-sided ACM phenotypes. Data regarding physical exercise was not routinely collected for patients enrolled in the study cohort and therefore no specific sub-analysis regarding the impact of physical exercise on VA events in patients with a DSP-ACM could be performed.

CONCLUSIONS

Patients with DSP-ACM are at particularly high risk for VA events. While arrhythmic risk markers for DSP-ACM partially overlap with those of classical ACM, patients with female sex were at similar, if not higher, risk, and age was a less informative marker. The ARVC risk calculator had poor performance in patients with DSP-ACM, albeit better in those patients with right sided disease. Patients with DSP-ACM may benefit from the development of gene-specific risk stratification tools. In the meanwhile, the ARVC risk calculator should only be used in DSP-ACM patients who both meet 2010 TFC and have no LV involvement. In the remaining patients evidence of ventricular arrhythmias (NSVT, PVC count) are salient markers of risk.

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SUPPLEMENTARY MATERIALS

Table S1

# of individuals	Deoxyribonucleic acid change	Amino acid change	Secondary Pathogenic Variant
1	c.3G>T	p.M1I	
1	c.37_38delAC	p.T13Sfs*79	
1	c.151C>T	p.Q51*	
1	c.170+1G>T	Splice site	
2	c.250C>T	p.R84*	
1	c.273+3A>G	Splice site	
1	c.305C>T	p.R1269*	
1	c.356dupA	p.I120Nfs*16	
3	c.448C>T	p.R150*	
1	c.478C>T	p.R160*	*MYBPC3* deletion exons 13-22
8	c.478C>T	p.R160*	
4	c.699G>A	p.W233*	
2	c.818_819insA	p.N274Efs*15	
1	c.825_827delCAT	p.I276del	
1	c.859A>C	p.N287H	
2	c.861T>G	p.N287K	
1	c.872_873dup	p.Q292Rfs*26	
4	c.877G>A	p.E293L	
2	c.878A>T	p.E293V	
1	c.888C>G	p.Y296*	
4	c.939+1G>A	Splice Site	
1	c.939+2T>C	Splice Site	
1	c.946_947insATACGCA	p.M316fs	
1	c.967G>C	p.E323Q	*DSP* c.692A>G p.Y231C
1	c.1048T>C	p.Y350H	
1	c.1054_1059delinsCA	p.D352Hfs*20	
3	c.1060_1061delCT	p.L354Afs*15	
1	c.1067C>A	p.T356L	
1	c.1080G>T	p.W360C	
1	c.1141-2A>T	Splice site	
4	c.1146delT	p.F382fs	
2	c.1209C>G	p.Y403*	
1	c.1266+1G>T	Splice site	
2	c.1267-1A>G	Splice site	
1	c.1293del	p.Y431*	

1	c.1325C>T	p.S442F	*PKP2* c.2146-1G>C
2	c.1339C>T	p.Q447*	
1	c.1351C>G	p.R451G	
2	c.1351C>T	p.R451C	
1	c.1419+1G>A	Splice site	
3	c.1520C>T	p.S507F	
1	c.1582C>T	p.Q528*	
5	c.1691C>T	p.T564I	
1	c.1705A>T	p.K569*	
2	c.1754_1755insA	p.L585fs	
2	c.1758T>A	p.H586Q	
2	c.1762C>T	p.Q588*	
1	c.1790C>T	p.S597L	
1	c.1816C>T	p.R606W	
2	c.1831C>T	p.Q611*	
2	c.1873C>T	p.Q625*	
1	c.1891C>T	p.Gln631*	
1	c.1911T>A	p.D637E	
1	c.1938C>A	p.C464*	
2	c.2063_2064delTC	p.L688Efs*19	
2	c.2297+2T>A	Splice site	
2	c.2390_2393del	p.V797fs	
1	c.2437-1G>C	Splice site	
1	c.2497C>T	p.Gln833*	
1	c.2509delA	p.S837Vfs*94	
1	c.2533C>T	p.Q845*	
1	c.2631-2A>C	Splice site	
1	Large duplication starting at c.2631-?_c.8616+?dup		
1	c.2643_2646dup	p.K883Gfs*12	
1	c.2650C>T	p.Q884*	
9	c.2821C>T	p.R941*	
1	c.2848del	p.Il950Lfs*27	
2	c.2848del	p.I950Lfs*976	PKP2 p.R480Lfs*499
1	Large duplication including		
1	c.2909C>G	p.S970*	
1	c.3022dup	p.T1008Nfs*12	
2	c.3045delG	p.S1015fs*	
2	c.3049_3050dupTT	p.L1017Ffs*2	
3	c.3133C>T	p.R1045*	
1	c.3160_3166del10	p.K1054Sfs*26	

1	c.3160_3169delAAGAACAA	p.K1054fs	
1	c.3203_3204delAG	p.E1068Vfs*19	
6	c.3337C>T	p.R1113*	
1	c.3416A>G	p.Y1139C	
1	c.3434delC	p.A1145fs*14	
2	c.3465G>A	p.W1155*	
1	c.3474_3475insA	p.E1159Rfs*3	
2	c.3474dupA	p.E1159fs	
1	c.3505T>A	p.Y1169N	
1	c.3526delG	p.V1176fs	
1	c.3541G>T	p.Q1181*	
2	c.3679C>T	p.Q1227*	
1	c.3724_3739delCTTCAAGGGAAAATC	p.L1242Efs*3	
1	c.3735_3741dupAAATCGA	p.D1248Lfs*7	
1	c.3739C>T	p.R1247*	
1	c.3784C>T	p.Q1262*	
3	c.3793G>T	p.E1265*	
2	c.3799C>T	p.R1267*	
1	c.3928A>T	p.K1310*	*PKP2* c.2578-3A>G
1	c.3928A>T	p.K1310*	
1	c.3946delT	p.Q1311Pfs*13	
1	c.4003C>T	p.Q1335*	
1	c.4180C>T	p.Q1394*	
1	c.4198C>T	p.R1400*	
1	c.4333C>T	p.Q1445*	
2	c.4477G>T	p.E1493*	
1	c.4518delA	p.R1506fs*	
5	c.4531C>T	p.Q1511*	
1	c.4608_4612del	p.R1537Efs*5	
1	c.4711C>T	p.Q1571*	
1	c.4756_4757del	p.E1586Rfs*40	
2	c.4797delA	p.G1600Afs*2	
1	c.4824dupA	p.A1609Sfs*18	
1	c.4875delT	p.K1626Rfs*19	
1	c.5014C>T	p.Q1672*	*PKP2* deletion exons 5-7
1	c.5208_5209del	p.G1737Tfs*7	
5	c.5212C>T	p.R1738*	
2	c.5342delG	p.R1781Nfs*12	
1	c.5419C>T	p.Q1807*	
1	c.5472delA	p.D1825Wfs*12	*LMNA* c.1711_1712delCGinsTC, p.Arg571Ser

2	c.5596C>T	p.Q1866*	
1	c.5659_5660delAA	p.K1887fs	
1	c.5800C>T	p.R1934*	
1	c.5806C>T	p.Q1936*	
3	c.5851C>T	p.R1951*	
2	c.6273delA	p.A2092Lfs*24	
1	c.6336delG	p.N2114Ifs*2	
2	c.6348_6351delTGAT	p.D2117Efs*17	
2	c.6393delA	p.G2133Vfs	
1	c.6398dupG	p.V2134Cfs*22	
1	c.6456dupG	p.L2153Afs*3	
2	c.6478C>T	p.R2160*	
1	c.6496C>T	p.R2166*	
1	c.6504_6507del	p.S2168Rfs*18	
1	c.6553C>T	p.Q2185*	
1	c.6767G>A	p.G2256D	
1	c.6850C>T	p.R2284*	
1	c.6937delG	p.E2313Rfs*	
1	c.6954_6955delGG	p.G2319Sfs*5	
1	c.7000C>T	p.R2334*	
1	c.7066A>T	p.K2356*	
1	c.7096_7103delCGCTTATT	p.L2367Sfs*10	
2	c.7096C>T	p.R2366C	
1	c.7128del	p.I2377Sfs*14	
1	c.7180delA	p.R2394fs*	
1	c.7206_7209del	p.S2402Rfs*27	
1	c.7240G>T	p.G2414*	
1	c.7248dupT	p.D2417*	
1	c.7293_7296del	p.E2431Dfs*15	
3	c.7567_7570del	p.K2523Qfs*37	
1	c.7569_7573delACAG	p.T2524Afs*36	
1	c.7583>G	p.Y2528C	*PKP2* c.1237C>T, p.R413*
1	c.7968_7972delCACAG	p.C2656Wfs*24	
1	c.7999C> T	p.Q2667*	
1	c.8075_8078del	p.2692_2693del	*PKP2* c.2146-1G>C
2	c.8077_8080delAAAG	p.K2693Pfs*3	
1	c.8156del	p.P2719Rfs*27	
1	c.8188delC	p.Q2730Sfs*16	
1	c.8309A>G	p.Y2770C	
1	c.8392_8393del	p.T2798Wfs*53	
1	c.8462_8463del	p.S821Cfs*30	

1	c.8469_8487delGGGGTCCCGCTCCGGCTCC	p.G2824fs	
1	c.8471_8483delGGTCCCGCTCCGG	p.G2824Afs*55	
1	deletion 6p24.1p25 encompassing DSP	deletion 6p24.1p25 encompassing DSP	*PKP2* c.2197_2202delCACACCinsG *p.His733Alafs*8*
2	deletion 6p24.1p25 encompassing DSP	deletion 6p24.1p25 encompassing DSP	

Table S2

5-yr arrhythmic risk predictors						
	Univariate			Multivariate		
	HR	95% C.I.	p	aHR	95% C.I.	p
Age (/year)	0.998	0.984–1.011	0.723			
Male sex	0.710	0.420–1.199	0.200			
TWI tot (/lead with TWI)	0.960	0.868–1.061	0.423			
PVC burden (log)	1.189	1.034–1.368	**0.015**	1.089	0.940–1.261	0.254
Cardiac Syncope	1.038	0.532–2.026	0.912			
History of NSVT	2.629	1.655–4.176	**<0.001**	2.249	1.374–3.682	**0.001**
RVEF (/%)	0.978	0.960–0.998	**0.027**	0.985	0.953–1.005	0.141
LV involvement	1.391	0.816–2.373	0.225			

Table S3

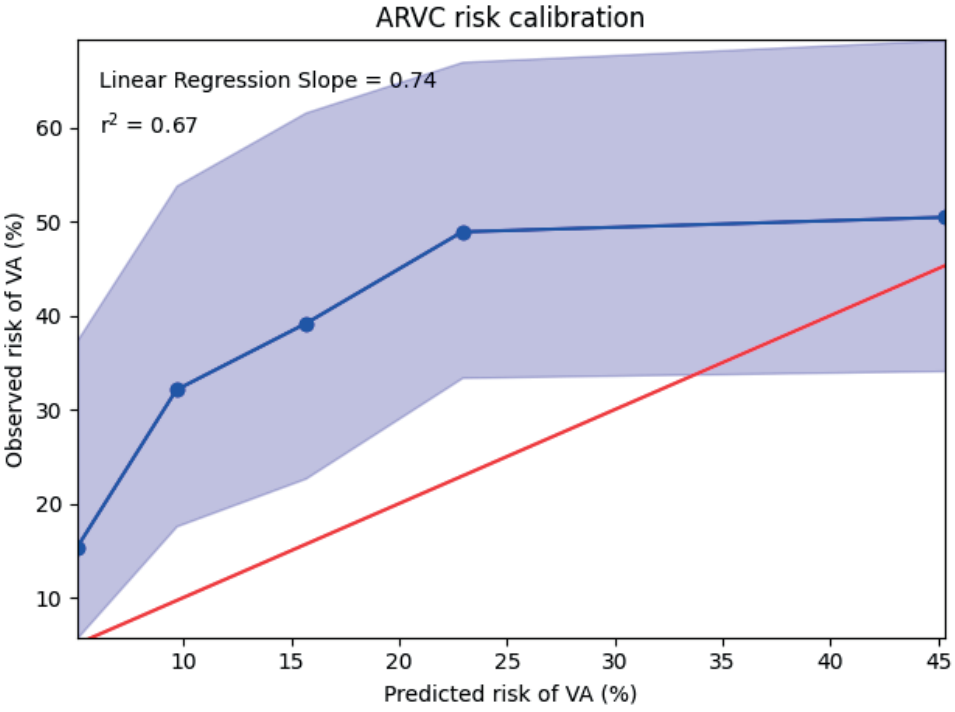
Overall

5-yr arrhythmic risk predictors						
	Univariate			Multivariate		
	HR	95% C.I.	p	aHR	95% C.I.	p
Age (/year)	0.998	0.984–1.011	0.723			
Male sex	0.710	0.420–1.199	0.200			
TWI tot (/lead with TWI)	0.959	0.867–1.061	0.417			
PVC burden (log)	1.207	1.047–1.392	**0.010**	1.045	0.896–1.219	0.575
Cardiac Syncope	1.038	0.532–2.026	0.912			
History of NSVT	2.629	1.655–4.176	**<0.001**	2.236	1.364–3.663	**0.001**
RVEF (/%)	0.981	0.962–0.999	**0.046**	0.997	0.975–1.019	0.768
LVEF (/%)	0.969	0.953–0.986	**<0.001**	0.977	0.956–0.998	**0.028**
LV LGE	1.450	0.830–2.533	0.192			

Table S4
Primary Prevention

5-yr arrhythmic risk predictors						
	Univariate			**Multivariate**		
	HR	95% C.I.	p	aHR	95% C.I.	p
Age (/year)	0.998	0.983–1.013	0.805			
Male sex	0.555	0.293–1.050	*0.070*	0.613	0.322–1.169	0.138
TWI tot (/lead with TWI)	0.960	0.857–1.074	0.473			
PVC burden (log)	1.258	1.076–1.471	**0.004**	1.137	0.952–1.357	0.156
Cardiac Syncope	0.734	0.315–1.712	0.474			
History of NSVT	2.506	1.491–4.213	**<0.001**	1.643	0.916–2.947	*0.096*
RVEF (/%)	0.982	0.960–1.004	0.108			
LVEF (/%)	0.969	0.951–0.988	**0.001**	0.983	0.960–1.006	0.143
LV LGE	2.187	1.146–4.172	**0.018**	1.653	0.823–3.321	0.158

Figure S1



Calibration plot for the performance of the ARVC Risk Calculator in the overall study cohort.

Figure S2

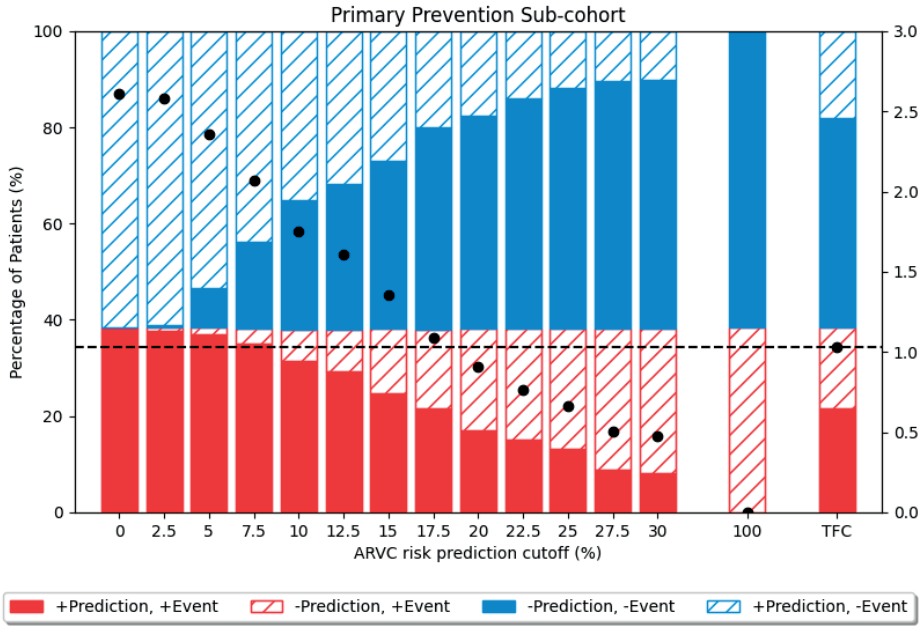


Figure S2 shows the rates of appropriate versus inappropriate ICD implantation that were achieved with current clinical practice (TFC) and that would have been achieved using different ARVC risk calculator-based 5-year VA risk thresholds in the primary prevention sub-cohort. In each column, red cells (full bins + striped bins) represent the percentage of patients experiencing an event during the 5 year follow up. Blue cells (full bins + striped bins) represent the percentage of patients not experiencing an event during the 5 year follow up. Full red bins represent patients experiencing a VA event with an ICD (true positives). Striped red bins represent patients experiencing a VA event without the protection of an ICD (false negatives). Full blue bins represent patients not experiencing an event and without receiving an ICD (true negatives). Striped blue bins represent patients not experiencing a VA event receiving an ICD (false positives).The black dot represents of ICD implanted : VA events observed within a column. The dotted line represents the reference level of ICD implanted : VA event ratio observed according to current clinical practice (TFC) implantation indications.

Figure S3

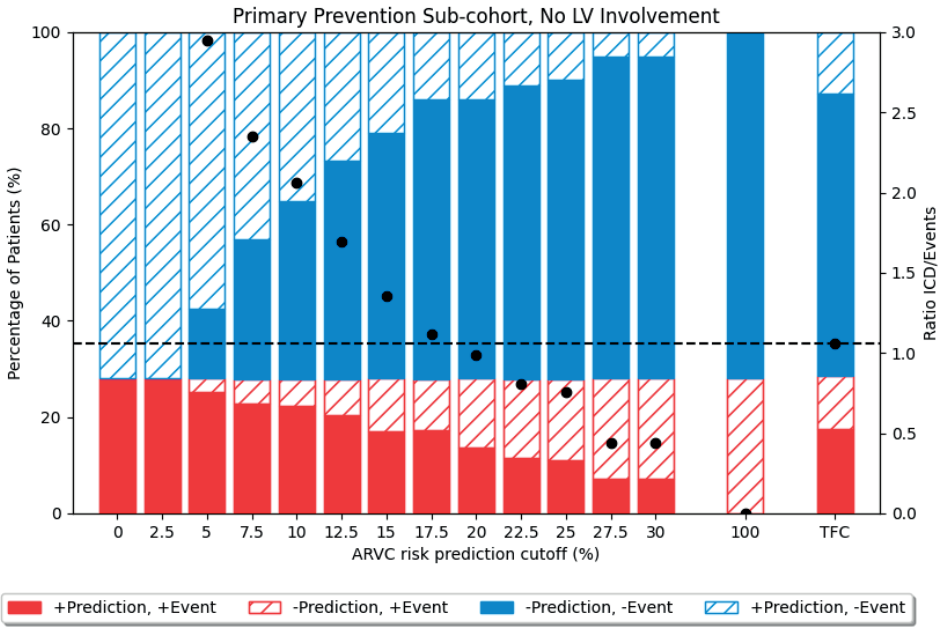


Figure S3 shows the rates of appropriate versus inappropriate ICD implantation that were achieved with current clinical practice (TFC) and that would have been achieved using different ARVC risk calculator-based 5-year VA risk thresholds among patients in the primary prevention sub-cohort without LV involvement. In each column, red cells (full bins + striped bins) represent the percentage of patients experiencing an event during the 5 year follow up. Blue cells (full bins + striped bins) represent the percentage of patients not experiencing an event during the 5 year follow up. Full red bins represent patients experiencing a VA event with an ICD (true positives). Striped red bins represent patients experiencing a VA event without the protection of an ICD (false negatives). Full blue bins represent patients not experiencing an event and without receiving an ICD (true negatives). Striped blue bins represent patients not experiencing a VA event receiving an ICD (false positives).The black dot represents of ICD implanted : VA events observed within a column. The dotted line represents the reference level of ICD implanted : VA event ratio observed according to current clinical practice (TFC) implantation indications.

Figure S4

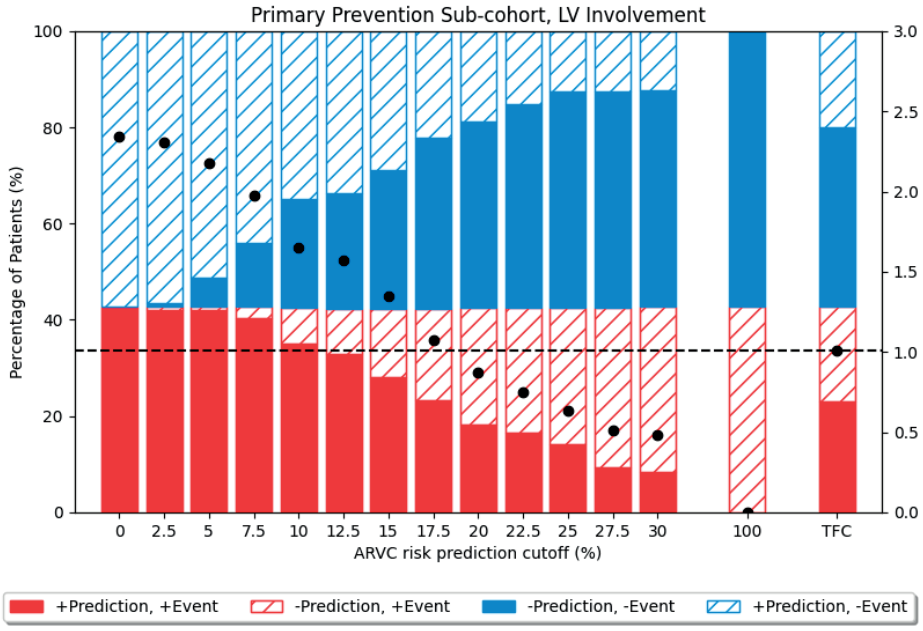


Figure S4 shows the rates of appropriate versus inappropriate ICD implantation that were achieved with current clinical practice (TFC) and that would have been achieved using different ARVC risk calculator-based 5-year VA risk thresholds among patients in the primary prevention sub-cohort with LV involvement. In each column, red cells (full bins + striped bins) represent the percentage of patients experiencing an event during the 5 year follow up. Blue cells (full bins + striped bins) represent the percentage of patients not experiencing an event during the 5 year follow up. Full red bins represent patients experiencing a VA event with an ICD (true positives). Striped red bins represent patients experiencing a VA event without the protection of an ICD (false negatives). Full blue bins represent patients not experiencing an event and without receiving an ICD (true negatives). Striped blue bins represent patients not experiencing a VA event receiving an ICD (false positives).The black dot represents of ICD implanted : VA events observed within a column. The dotted line represents the reference level of ICD implanted : VA event ratio observed according to current clinical practice (TFC) implantation indications.

CHAPTER 10

SUMMARY AND FUTURE DIRECTIONS

The studies included in this thesis aim to provide validation for the original ARVC Risk Calculator algorithm for incident ventricular arrhythmic risk stratification in patients with ARVC, in order to support its use in the clinical setting. This thesis also provides data refining the performance of the original risk stratification tool, by describing Bayesian implementation of results from programmed ventricular stimulation procedures as well as the use of multiple tests during follow-up for continuous longitudinal updating of the calculator risk estimations. Finally, this work tackles the under-described topic of risk stratification in non-classical, left-dominant ARVC phenotypes and the performance of the ARVC risk calculator in such patients. This final chapter summarizes the main findings of this thesis and outlines possible directions for future research in these areas.

PART I – VALIDATING THE ARVC RISK CALCULATOR

Sudden cardiac death (SCD) events and malignant ventricular arrhythmias (VAs) represent the biggest threat that patients diagnosed with ARVC face. Modern medical knowledge unfortunately still falls short of a causative treatment for this disease. Current guidelines indicate the placement of an implantable cardioverter defibrillator (ICD) as the cornerstone for SCD reduction and for disease management(1–3). Therefore, once the diagnosis of ARVC is made, the next step in clinical management is to assess whether or not the placement of an ICD is necessary. The benefits associated with ICDs in this setting are self-evident and the placement of such devices often provides a sense of security to both ARVC patients and their managing physicians. Recommending an ICD in every patient with ARVC, given the theoretical increased risk of SCD, may therefore at first glance seem a reasonable option. ICD placement, however, always comes at a cost: although a routine surgical procedure, it is still an invasive procedure, potentially associated with patient discomfort and procedural complications. Furthermore, patients diagnosed with ARVC are usually young and will carry an ICD for life, and thus will be particularly prone to device-associated complications (i.e. lead malfunctions, infections, inappropriate device therapies) as well as potential psychological trauma and body image issues(4–6). As the clinical course of individual patients with ARVC varies widely, correct identification of which patients would truly benefit from ICD implantation is often challenging. While there is overwhelming consensus for the benefits of offering ICDs to patients with ARVC and previous episodes of sustained VA(1–3), the indications for primary prevention ICD placements in patients with ARVC have been extensively debated. Studies assessing the performance of these ICD placement indications, in fact, have reported limited effectiveness in risk stratification among patients without previous VA: up to two-thirds of primary prevention ICDs placed in patients with ARVC did not deliver therapy at long term follow up, resulting in a high number of ICDs

implanted per sustained VA treated and probably indicating an excessively cautious and aggressive treatment approach by the ARVC community(6–10).

For these reasons, the development in 2019 of the ARVC Risk Calculator tool for primary prevention ICD guidance for patients with ARVC by a multicenter collaboration was saluted as a major scientific achievement of the field. In the derivation cohort in fact, the risk estimations obtained from the ARVC Risk Calculator were more precise than all other available risk stratification strategies(9). Additionally, authors reported that an ICD placement strategy based on this tool's estimates would have led to a very high protection from SCD associated with a significant reduction in ICD placement rates (-20.3%), with a significant improvement in the ratio of ICD placed for arrhythmic event treated. While looking extremely promising, the development of this tool was however only the first step in a lengthy journey towards its full acceptance by the scientific community.

Early validation experiences

The scientific method requires the hot-blooded enthusiasm associated with novel promising discoveries to undergo the cold scrutiny of the process of external replication and validation, in an elaborate dance composed of due process, checks and balances. In medicine, this process may be lengthy and difficult, especially in the context of a relatively rare disease such as ARVC.

Chapter 3 of this thesis represents the first step of the validation process for the ARVC Risk Calculator. In this study, we reported clinical outcomes from 101 consecutive patients with definite ARVC and an extensively characterized disease phenotype that was evaluated at a tertiary level electrophysiology center. The study cohort was at a high risk of arrhythmic events, with around 35% of patients experiencing sustained VA events at the 5-yr follow up mark. In the study, the ARVC Risk Calculator performed well, with a non-significant difference of 6.7 [–4.3; 17.7] % in calculator predicted vs empirically observed rates of arrhythmias over 5 years of follow up.

In the context of this thesis and in the bigger picture of ARVC risk stratification, this chapter is important for multiple reasons. First, although the original ARVC Risk Calculator was internally validated through bootstrap resampling(9), resampling validation strategies can only address sample size limitations, while remaining prone to inclusion criteria and selection biases. Hence, **Chapter 3** represents the first real world assessment of the performance of the ARVC Risk Calculator in an fully external ARVC cohort. It shows for the first time that real, independent patients with ARVC are adequately stratified by this novel tool, validating its effectiveness. A net-benefit analysis addressing how different 5-yr risk estimate thresholds for ICD implantation would have performed in terms of VA protection in this cohort closely resembled the results observed in the developmental cohort: the

ARVC Risk Calculator was superior to the 2015 TFC consensus for risk stratification, with a maximal net benefit associated with a bracket of 15-20% 5-yr predicted risk (vs the 12.5-17.5% 5-yr reported in the development cohort). The patient population baseline characteristics are an additional point of interest of this study. Compared to the population used for the development of the ARVC Risk Calculator, patients in this study were in fact more frequently probands (83.2% vs 49.8%) and males (75.3% vs 44.7%). Both those characteristics are associated with a higher arrhythmic risk profile in patients with ARVC, as demonstrated by the high VA event rate observed. This notwithstanding, the ARVC Risk Calculator performed exceedingly well in such a high risk cohort, increasing confidence in the algorithm and proving its generalizability in a first specific subset of ARVC patients (i.e. high risk patients) that may be seen by specific referral centers. Finally, **Chapter 3** described the arrhythmic risk of patients with non-classical ARVC phenotypes (i.e. biventricular or left-dominant ARVC), an historically under-described field of ARVC research. Non-classical phenotypes were reported as very arrhythmogenic, prone to significantly higher risk of arrhythmias than classical right-dominant phenotypes (5-yr freedom from VA 76% vs 58%). Through a sub-analysis of its performance in these specific non-classical phenotypes, this study identified one of the limitations of the ARVC Risk Calculator: namely, potential underprediction of arrhythmic risk in patients with non-classical biventricular or left-dominant ARVC phenotypes. This is discussed more extensively in the "Risk Stratification for Biventricular and Left Dominant Phenotypes" section below.

ARVC Risk Calculator and Physical Exercise

Physical exercise is an important risk factor associated with an increased occurrence of arrhythmic events in patients with ARVC(11) and a clear dose-response association between the extent of exercise exposure and the increase in arrhythmic risk has been described(12). As such, patients diagnosed with this condition are excluded from competitive sports participation and recommended to significantly reduce their training regimes. The original study introducing the ARVC Risk Calculator did not report any data addressing exercise exposure and athlete status of the included patients, immediately leading the community to wonder if the risk calculator findings would hold true in ARVC patients with a significant lifetime exercise exposure(13). **Chapter 4** addresses this question, reporting the first assessment of the performance of the ARVC Risk Calculator in a cohort of 25 high-end endurance athletes diagnosed with ARVC and therefore recommended to undergo clinical detraining. The study cohort was followed for a median of 5.3 years, with multiple repeated 24-h ECG Holter monitors and stress test ECGs collected during follow-up and after patient clinical detraining.

In this study cohort, an almost perfect overlap between Risk Calculator predicted and observed VA rates was reported (mean difference over 5-yr -0.85% [-4.8 to 3.1]). This finding has a clear impact on the clinical management of patients with ARVC: while it is in fact of paramount importance to educate patients and strongly recommend exercise restriction, **Chapter 4** supports the use of the ARVC Risk Calculator as a risk stratification strategy regardless of a patient's exercise status. Physical exercise is an indisputable additional risk factor for arrhythmias in patients with ARVC, but the clinical parameters included in the ARVC Risk Calculator seem to already account for the increased arrhythmic risk to which athletes are exposed. This may be explained by athletes with ARVC having in fact lower RVEF values, higher 24-h PVC burdens, and more negative T wave inversions at the 12 lead ECG than patients with ARVC who are not athletes, leading to a higher ARVC Risk Calculator predicted arrhythmic risk. The findings in **Chapter 4** represented the basis for a subsequent study from *Bosman* et al, that 2 years later confirmed these findings in a larger sample size of 176 ARVC patients for which an exercise interview was available(14). Even in that larger patient cohort, adding a correction for exercise exposure did not significantly improve the performance of the original ARVC Risk Calculator. Following these two studies, the current version of the ARVC Risk Calculator is considered adequate for risk stratification in both athletes and non-athletes diagnosed with ARVC.

Chapter 4, however, also provided additional insights into the complex interaction between physical exercise and the natural history of ARVC. In this study, a strong reduction in the PVC burden (median reduction -1682 [-536; -2828] PVC/24h) and a significant improvement in the amount of arrhythmias observed during stress test ECG assessments of patients after undergoing clinical detraining were observed. The decrease in PVC burden was most significant within the first 6 months after the start of clinical detraining, while the overall PVC burden plateaued at around 18 months of complete exercise detraining. These improvements in the arrhythmic phenotype of disease were independent from the use of beta-blockers and other anti-arrhythmic drugs. The dynamic changes in PVCs described in this study have great importance for longitudinal risk stratification strategies at each follow up reassessment. In particular, the observation that the electrical remodeling achieved through clinical detraining peaked at 18 months, with a PVC burden remaining stable thereafter, has been the rationale upon which several of those risk stratification strategies have been based. More about this topic will be discussed in **Chapter 7** and **Chapter 8**, summarized in the section "*Longitudinal Risk Stratification Strategies*" below.

Full Scale Validation for the ARVC Risk Calculator

The experiences reported in **Chapter 3** and **Chapter 4** represent only the beginning of the vast efforts that the entire ARVC community started in 2019 aiming at the full validation of the ARVC Risk Calculators. Immediately after the publication of the studies presented in Chapter 3 and 4, in fact, *Aquaro* et al, *Baudinaud* et al, and *Zhang* et al all reported similar results at a single center level(15–17). All those studies further confirmed the effectiveness of the novel risk stratification tool, with results particularly adequate for the risk stratification of patients with classical, right-sided ARVC phenotypes. This combined body of evidence, however, still fell short of the adequate sample size required to fully validate the ARVC Risk Calculator beyond any reasonable doubt.

To overcome this problem and achieve a complete validation of the tool, a dedicated and adequately powered external validation study was then planned, under the leadership of those centers that developed the original ARVC Risk calculator. **Chapter 5** describes this effort, reporting on the performance of the ARVC Risk Calculator in a cohort of 429 patients with ARVC obtained by involving 29 centers from 6 countries in Europe and in North America. Over a median follow-up of 5.02 years, 24% of patients experienced a first sustained VA (event rate of 4.98 [4.07–6.04] %/year]). With 103 events recorded, the study exceeded the pre-specified threshold of 100 events required for an adequately powered model validation. In this external cohort, the ARVC Risk Calculator performed well in terms of arrhythmic risk stratification, with a C statistic of 0.70 (0.65 – 0.75), and no sign of under- or over- prediction across the entirety of the included patient spectrum.

In a truly successful story for the whole ARVC community and for scientific integrity, a similarly powered, completely independent validation study was published at the same time by *Protonotarios* et al(18). Most of the centers involved in this second study focus more on heart failure than on cardiac arrhythmias. The ARVC patient type addressed by *Protonotarios* et al could have potentially been different from those presented in **Chapter 5**, as most of the involved centers were heart failure or cardiomyopathy-led clinics instead arrhythmia and cardiac electrophysiology units. This potential difference was indeed reflected in an overall lower yearly event rate (2.6 [1.9–3.3] %/year) of sustained arrhythmias. Nonetheless, the performance of the ARVC Risk Calculator in the *Protonotarios* et al cohort yielded very similar results to the one reported in **Chapter 5** (overall C statistic of 0.75 [0.70–0.81]). **Chapter 5**, in combination with the additional results reported by *Protonotarios* et al, therefore presents the ARVC community with statistically-reliable evidence that the ARVC Risk Calculator is an adequate tool for arrhythmic risk stratification for patients with ARVC across cardiomyopathy and cardiac arrhythmia units.

In light of the existence of several different guidelines for the placement of primary prevention ICDs in patients with ARVC, in **Chapter 5** we specifically tested the hypothesis that the ARVC Risk Calculator was a better risk stratification tool than all the other available risk stratification strategies (TFC 2015, AHA 2017, HRS 2019). Similar to what was observed in the original study introducing the ARVC Risk Calculator, an ICD stratification tool based on this tool using any threshold < 35% of 5-yr predicted risk for ICD recommendation presented a higher net clinical benefit than all other available guidelines. Hence, these findings confirm the idea that by using the ARVC Risk Calculator a similar protection rate from sustained ventricular arrythmias with a significantly lower number of ICD placed per ventricular arrhythmia treated can be achieved. Similar to what was reported in previous studies(9,19), the clinical net-benefit is maximal when a threshold between 5% and 25% of 5-yr predicted risk is used at no increased cost in terms of protection rates. The ARVC Risk Stratification tool, however, should be viewed as a tool that can provide reliable data to facilitate shared decision-making in regards for ICD implantation. No recommendation for an absolute threshold for ICD implantation is provided, as those risk estimates, as well as the clinical benefit, should always be weighted with the set of values and preferences of each individual patient. Of note, new guidelines for the management of patients with VA were published by the European Society of Cardiology in 2022 (3). The potential performance of those new guidelines was not tested in the study reported in **Chapter 5**, as they were published after the completion of the study. However, as the indications for primary prevention ICD implantation in ARVC patients do not seem significantly different from the previous guidelines, a better performance in risk stratification of the ARVC Risk Calculator seems very plausible.

With these considerations, **Chapter 5** concludes **Part I** of this PhD Thesis, providing readers with evidence backing the indication of the ARVC Risk Calculator as the modality of choice for arrhythmic risk stratification in patients with ARVC and no previous sustained ventricular arrythmias. While currently presenting it as the most effective risk stratification modality, **Part I** also identifies and discusses some areas of potential improvement for the ARVC Risk Calculator. **Chapter 3** showed a potential underestimation of the arrhythmic risk in patients with a non-classical phenotype. The work of *Protonotarios* et al highlights poorer risk stratification performance in their patients with a gene-elusive or non-classical desmosomal genotype (i.e. desmoplakin variant carriers). **Part II** of this thesis addresses this in greater detail and tries to overcome some of these limitations, aiming at the improvement of that model that was validated and widely accepted through the efforts of **Part I.**

PART II – REFINEMENT OF THE ARVC RISK CALCULATOR

Since the introduction of the ARVC Risk Calculator, it was immediately clear that several other potential predictors not yet addressed or included in the original tool could be of potential interest for additional risk stratification refinements(13). Part II of this thesis describes several incremental efforts that have been undertaken to improve the performance of the ARVC Risk Calculator and to fully integrate its use into the clinical workflow for ARVC patient management.

Programmed Ventricular Stimulation for Risk Stratification in ARVC

The role of programmed ventricular stimulation for arrhythmic risk stratification in patients with ARVC is a controversial topic. Over the years, multiple studies have tried addressing this topic: although some studies supported the role of PVS as a strong predictor of sustained VA in ARVC(20,21), others have reported a poor positive predictive value of this test for arrythmias during follow up(22). All these studies, however, have been hampered by a limited sample size. Furthermore, those prior studies grouped patients with ARVC with and without previous arrhythmic events together, without considering the potentially different yield that PVS results could have in these different subpopulations. Finally, in the post ARVC Risk Calculator era, it has been unclear how the findings and risk estimates derived from the risk calculator and PVS results interact with each other in optimal risk stratification of patients with ARVC referred for the evaluation of the need for a primary prevention ICD placement.

With **Chapter 6,** we tried to address these unsolved questions leveraging data from a large, multinational cohort of 288 patients with ARVC with no prior history of sustained VA undergoing PVS. Around half of the cohort was inducible at PVS and a very strong association between the inducibility status of a patient during PVS and an increased (roughly a 4-fold) arrhythmic risk during follow-up was observed. These findings confirmed the results reported by *Saguner* et al and expanded the preliminary findings of **Chapter 3,** both showing strong association between PVS status and long-term outcomes but in cohorts with a sample size too small to allow for definite answers. To allow full integration of the PVS results with the ARVC Risk Calculator, in **Chapter 6** we additionally performed a Bayesian analysis, using positive and negative likelihood ratios associated with the results from PVS (LR+: 2.3; LR-: 0.36, respectively) to update the a-priori estimated risk derived from the ARVC Risk Calculator into a post-PVS refined risk. The performance of a multivariable model including both the ARVC Risk Calculator and the results from PVS was shown to be superior to either of the individual component in regards to the prediction of arrhythmic events at long-term follow-up. As such, the online website of the ARVC Risk Calculator has been updated accordingly to permit automatic calculation of post-PVS refined risk in eligible patients. Of note, we do not

believe that the performance of a PVS is needed in all patients diagnosed with ARVC. Unfortunately, the retrospective nature of the study inherently prevented us from identifying which patients should be referred for a PVS, as the indication for PVS was the independent clinical judgement of managing physicians and not a shared per-protocol algorithm. At the same time, however, **Chapter 6** clearly suggests a high informative value associated with results of PVS in patients with ARVC that are referred for PVS after a real-world clinical evaluation. In particular, the maximal clinical benefit of PVS was shown for patients with a low- to intermediate ARVC Risk Calculator derived a priori arrhythmic risk (≤ 25% risk at 5-yr). In this subset of patients, PVS yielded a fairly high negative predicted value (NPV: 92.7%) and a negative inducibility status could be used as a strong argument against the need for an ICD. With results now clearly proving that PVS results are associated with arrhythmic outcomes in ARVC and the complete integration between the two risk stratification tools, the final next step required is a prospective study assessing an ICD placement decision tree at the time of diagnosis based on ARVC Risk Calculator and PVS results.

PVC Changes & Longitudinal Risk Assessment in patients with ARVC

The ARVC Risk Calculator is a risk stratification tool meant for the assessment of the need for a primary prevention ICD at the time of disease diagnosis. ARVC, however, is a progressive disease and, as the individual risk of patients may significantly change over time, the task of patient management spans years of ongoing follow-up and surveillance. While progressive risk increases would not impact patients receiving an ICD at the time of disease diagnosis, as they are already protected by the implanted device, they could be of paramount importance for patients deemed at low arrhythmic risk at the time of first evaluation and therefore not receiving an ICD. At the time of the writing of this thesis, data on longitudinal assessments and dynamic risk stratification for the follow-up management of patients with ARVC was almost non-existent. Clinicians were left with only scarce evidence and no guideline-supported guidance on which follow-up strategy to adopt. **Chapters 7** and **Chapter 8** try to fill this evidence void and will be discussed together as they are strongly interconnected.

Chapter 7 describes how a strategy based on the use of repeated 24-h Holters in follow up is effective in dynamically tracking the arrhythmic risk of patients with ARVC. This study presents longitudinal data derived from a cohort of 169 patients followed up for a median of 4.5 years through multiple 24-h Holters performed yearly (total number of Holters: 723; median 4 Holters/patient). As preliminarily shown in **Chapter 4**, this study described a drop in the PVC burden immediately after disease diagnosis. This initial PVC burden drop, probably driven by clinical detraining and beta-blocker or anti-arrhythmic initiation at the of disease diagnosis, was followed by a phase of relative

stability in the PVC burden. At the time of any given Holter, the observed 24-h PVC burden was shown to be an effective estimate of the risk of sustained VA of the patient during the immediately following year. Within a framework of relative arrhythmic stability, sporadic episodes of year-to-year sudden PVC burden increases (called "PVC spikes" and defined as an absolute increase ≥5000 PVC or a relative increase ≥75%) were observed at an individual patient level. The observation of a PVC spike at a follow-up Holter was strongly associated with an increased (6-fold) arrhythmic risk in the 12 months immediately following that Holter. **Chapter 7** therefore presents findings suggesting that the changes in PVC counts and Holter parameters observed during patient follow up can be reliably used to dynamically track the longitudinal arrhythmic risk of patients with ARVC. A follow-up strategy employing Holter monitors is postulated as an effective way to constantly reassess those patients not receiving an ICD at the time of disease diagnosis, allowing for arrhythmic risk reevaluation and potentially leading to a new conversation regarding the need for an ICD in case of significant changes in the individual arrhythmic risk over time. These findings are the starting point of **Chapter 8**, which presents a longitudinal, dynamic risk assessment strategy based on updating ARVC Risk Calculator estimates by using follow up examinations, among which Holter monitors represent one of the most common. In a cohort of 408 US and Dutch ARVC patients with extensive longitudinal characterization, the performance of an ARVC Risk Calculated assessment based on tests at disease diagnosis is shown to start decreasing around the third year of follow up. However, updating the ARVC Risk Calculator estimate by inputting the most recent cardiac tests, completely ameliorates this decrement in performance.

In the setting of chronic, long-term management of the arrhythmic risk of patients with ARVC, findings from these two chapters are crucial, as they prove that the use of the ARVC Risk Calculator as a dynamic tool is possible. They provide clinicians with strong evidence that follow-up cardiac examinations can be used to dynamically track the arrhythmic risk of patients with ARVC and can be integrated within a general, unified umbrella risk stratification strategy. The ARVC Risk Calculator is therefore shown as a risk stratification tool capable of encompassing all phases of clinical management, from the moment of disease diagnosis throughout follow up. In short, the risk of incident sustained VA can now be continuously summarized through a single risk stratification tool into an up-to-date numerical value that can be tracked over time. This risk estimate can be used for a patient-physician informed discussion about the best course of action to undertake at different stages of disease chronic management, dynamically allowing for the weighting an objective and constantly updated metric of risk with the patient individual set of values in an empowering shared decision-making framework.

Risk Stratification for Biventricular and Left Dominant Phenotypes

The final chapter of this thesis is dedicated to the assessment of a specific subset of patients with ARVC, namely those patients reaching a diagnosis of ARVC and carrying a pathogenic/likely pathogenic (P/LP) variant in the desmoplakin (*DSP*) gene. This patient population represents a very unique subset of patients, as the vast majority of patients with *DSP*-based ARVC develop either a biventricular or a left-dominant disease phenotype. These non-classical disease phenotypes have been only recently recognized and the body of evidence describing natural the history of disease and gene-specific arrhythmic predictors in those patients is severely limited(23–25). At the same time, these limited findings combined with clinical experience seem to suggest *DSP*-ARVC is a highly arrhythmogenic disease, potentially requiring a very low threshold for ICD implantation. In the context of this thesis on modern modalities of risk stratification for patients with ARVC, *DSP* variant carriers represent an important clinical conundrum. The clinical adequacy of the ARVC Risk Calculator use for patients carrying *DSP* variants, even when fully complying with the eligibility criteria for usage of the ARVC Risk Calculator (definite ARVC diagnosis, no prior arrhythmic events), is in fact not clear. Findings from **Chapter 3** had hypothesized a significant risk underprediction by the ARVC Risk Calculator of the arrhythmic risk in patients with biventricular or left-dominant phenotypes, such as the one commonly seen in patients with *DSP*-ARVC, while other studies have instead reported a risk overprediction in *DSP* variant carriers(18). All these findings, however, have been derived from small sample size cohorts, potentially very prone to selection and referral biases due to individual center expertise (i.e. a lower arrhythmic but higher heart failure risk observed in cohorts of patients managed by heart failure specialists).

Chapter 9 represents our characterization of patients with a *DSP* P/LP variant and with a ARVC phenotype. Thanks to a very large international consortium, we gathered the largest cohort of *DSP* carriers ever to be assembled, comprised of 252 patients from 18 institutions from 8 different countries. In this patient cohort, we assessed long term arrhythmic outcome, potential gene-specific sustained VA predictors, and the performance of the ARVC Risk Calculator. As expected, a very high rate (71.0%) of patients with *DSP*-ARVC presented with a disease phenotype involving the left ventricle. Over slightly less than 4 years of clinical follow up from the time of definite ARVC diagnosis, a very high rate of patients with a *DSP*-ARVC (37.3%) experienced a sustained VA event. Of great interest, even patients without an history of previous sustained VA events at the time of disease diagnosis faced high yearly rates of sustained VA events (7.7%/year). Those yearly rates of arrhythmic events were higher than those reported in similar cohorts composed mostly of classical plakophilin-2 carriers and/or gene-elusive ARVC patients (*Cadrin-Tourigny* et al: 5.6%/yr(9); **Chapter 5**: 4.98%/yr; *Protonotarios* et al: 2.6%/yr(18)), clearly confirming a high arrhythmogenicity

of the *DSP*-associated ARVC phenotype. Similar to classical ARVC, an analysis of the potential predictors of sustained VA events identified the presence of non-sustained ventricular tachycardia episodes at the time of disease diagnosis as very strong risk factor for future sustained VA events. Different from classical ARVC, female patients with *DSP*-ARVC did not have a lower arrhythmic risk than their male counterparts. Furthermore, in line with what was postulated in **Chapter 3,** for *DSP*-ARVC, the performance of the ARVC Risk Calculator in the overall cohort was poor, with a C statistic of 0.604 and a possible risk underprediction across the entire arrhythmic risk spectrum. Among patients without left ventricular involvement, performance of the ARVC Risk Calculator was better (C: 0.691 [0.678 - 0.704]), but some degree of risk underprediction was still present. For patients with a left ventricular involvement, instead, the ARVC Risk Calculator was completely inadequate (C. 0.561 [0.558 - 0.564]).

Findings in this chapter are of great importance for clinical management, as they deeply characterize the clinical profile of *DSP*-ARVC. The high event rate seen in this population is a call towards very active arrhythmic surveillance and an aggressive ICD implantation strategy. The best modality to perform risk stratification assessment in this population, however, still evades us. It seems clear that the current ARVC Risk Calculator is not the answer to this question. It is likely patients with *DSP*-ARVC would benefit from the development of *DSP*-specific risk stratification tools for the prediction of arrhythmic events, in a similar fashion to what has been successfully achieved for other gene-specific cardiomyopathies (i.e. *PLN*-cardiomyopathy)(26). Such an endeavor, however, will require time and extensive international collaborations. In the meantime, by extrapolating from our data, it would seem reasonable to recommend ICD implantation for patients with *DSP*-ARVC and episodes of NSVT, given the strong association of NSVT with complex VA events during follow up.

CONCLUDING REMARKS:

A precision medicine approach leveraging population-based big data to outline individual risk profiles for a patient tailored management represents the future of medicine. The ARVC Risk Calculator represents the embodiment of such an approach in the field of arrhythmic risk stratification for patients with ARVC. This validated tool has shown the best clinical yield for guidance of primary prevention ICD placement in patients with ARVC and has undergone several improvements since its publication, to allow integration with PVS and risk stratification updating during follow-up. This tool is now constantly used and well-integrated in the ARVC scientific community, as witnessed by the large amount of studies addressing it and by the more than 20,000 individual visits reported by the ARVC Risk Calculator website www.arvcrisk.com.

While impressive results have been achieved, this journey has yet to reach its final destination. A potential limit of the ARVC Risk Calculator is represented by the fact that, differently from other

cardiomyopathies such as hypertrophic cardiomyopathy, a high percentage of the VA encountered in patients with ARVC are stable ventricular tachycardia events, that may have lesser clinical importance and may represent a relatively weak proxy for SCD event. A recent study comprised of 864 patients with ARVC presented a risk stratification algorithm focused at predicting only "life threatening VAs" (defined as a composite of sustained VA faster than 250 bpm, VF, and SCD) in patients with ARVC (27). This second risk calculator was shown effective in both primary and secondary patients with ARVC and it potentially integrates effectively with the main ARVC Risk Calculator in the clinical management of patients with ARVC. Its external validation, however, will be required before it can be extensively implemented in routine clinical management.

Furthermore, the data reported in this thesis, alongside the experiences from other international groups, have shown how the current risk stratification algorithm does not seem to perform well in patients with ARVC and a *DSP* variant. The growing understanding of the genetic architecture of this disease, alongside with the increased availability of genetic testing, have taught us the undeniable link between genetic architecture and long term outcomes in patients with ARVC(18,28). It seems therefore logical to envision in the future of the ARVC research field a shift towards gene-based (or even variant type based) risk stratification strategies. **Chapter 9** represents our own attempt to start a conversation about *DSP* variant carriers. A larger cohort of *DSP* variant carriers is currently being assembled in order to provide the statistical power needed to develop a *DSP*-specific version of the ARVC Risk Calculator. This is however merely the beginning of what will be a large (and probably lengthy) research effort: to reach the next level of individualized patient management, a proper assessment of the relative gene-specific predictors for arrhythmias of each one of the major genes associated with the entire spectrum of the arrhythmogenic cardiomyopathy phenotype will be required, with novel analysis modalities (i.e. application of machine learning to ECG, echo or CMR) potentially improving the overall prediction performance of these models.

Finally, it is worth remembering that, while a tool of great importance and clinical utility, the ARVC Risk Calculator represents one only of the many pieces of the very complicated puzzle of ARVC research. The role of physical exercise on disease progression, the prognostic impact of harboring P/LP variants in multiple genes or in different regions of the same ARVC associated genes, or the relevance of sex hormones on phenotype development are just many potential examples of aspects of this disease that are not yet completely understood. Given the relative rarity of those patients, strong international collaborations of multiple expert consortia will continue to be crucial to address these and many other clinically relevant questions in the field of ARVC, in order to pursue as the ultimate goal the prevention of SCD among young, apparently healthy individuals using the lowest achievable number of ICDs.

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APPENDIX

NEDERLANDSE SAMENVATTIG

Aritmogene rechter ventrikel cardiomyopathie (ARVC) is een erfelijke hartspierziekte (cardiomyopathie) die 1 op de 5000 mensen in Europa treft. De prevalentie van de ziekte varieert echter aanzienlijk tussen de verschillende landen als gevolg van genetische geografische clustering, waarbij Nederland, Noord-Italië en Griekenland het hoogste aantal gemelde gevallen binnen de Europese Unie hebben. ARVC is gekarakteriseerd door progressieve vervangingen van myocardcellen door vet en fibreus weefsel, voornamelijk in de rechter kamer van het hart. Deze pathogene weefselverandering vormt een groot probleem omdat dit het risico verhoogt van plotselinge en gevaarlijke hartritmes, die ventriculaire aritmieën (VA's) worden genoemd. Deze kunnen symptomen veroorzaken als hartkloppingen, licht in het hoofd en flauwvallen, en in sommige gevallen kunnen ze leiden tot cardiovasculaire collaps en zelfs plotselinge hartdood. ARVC is ook bijzonder zorgwekkend omdat het jonge, actieve en ogenschijnlijk gezonde mensen in hun twintiger en dertiger jaren treft.

Helaas bestaat er geen genezing voor ARVC, dus moeten artsen focussen op het behandelen van het natuurlijke beloop van de ziekte. Een manier om het risico op plotse hartdood te verminderen is om een implanteerbare cardioverter defibrillator (ICD) te implanteren. Dit apparaat geeft elektrische therapie en schokken af aan het hart om te voorkomen dat gevaarlijke hartritmes schade veroorzaken. Het plaatsen van een ICD is echter invasief en kan fysieke en emotionele stress veroorzaken voor patiënten. Bovendien lopen patiënten met een ICD het risico op complicaties op lange termijn die verband houden met de ICD, zoals infecties en onterechte schokken. Daarom moeten de voor, -en nadelen zorgvuldig worden afgewogen voordat de beslissing om een ICD te implanteren wordt genomen.

Om te bepalen welke patiënten een ICD nodig hebben voeren artsen, ten tijde van de ARVC-diagnose, een proces uit dat aritmogene risicostratificatie wordt genoemd. Dit proces omvat de beoordeling van het individuele risico van een patiënt om VA's te ontwikkelen. Deze beoordeling wordt uitgevoerd met gespecialiseerde klinische algoritmen die rekening houden met factoren zoals de leeftijd en het geslacht van de patiënt en gegevens van klinische tests zoals elektrocardiogrammen (ECG’s), Holter ECG's en magnetic resonance imaging (MRI) beeldvorming. Tot voor kort was het belangrijkste instrument voor deze beoordeling een algoritme van de 2015 Task Force for ARVC Management Consensus. Een meer recenter algoritme genaamd de ARVC Risk Calculator heeft echter aangetoond beter te presteren in het voorspellen van het VA-risico en het

begeleiden van ICD-plaatsing. Dit algoritme is gebaseerd op een samenwerking tussen ARVC-onderzoeksgroepen in Europa en de Verenigde Staten, en het kan het individuele 5-jaars risico van een patiënt op VA schatten met behulp van routinematig verzamelde klinische variabelen. In vergelijking met het vorige algoritme is gebleken dat de ARVC Risk Calculator preciezer is bij het schatten van het VA-risico en het begeleiden van de ICD-plaatsing, terwijl het plaatsen van onnodige ICD's bij patiënten met een laag risico wordt verminderd.

Voordat de ARVC Risk Calculator echter op grote schaal kan worden gebruikt, moet het een extern validatieproces ondergaan. Bovendien zijn er sinds de eerste introductie verschillende gebieden geïdentificeerd die voor verbetering vatbaar zijn, waaronder het aanpakken van de rol van lichaamsbeweging op risicostratificatie, hoe om te gaan met risicostratificatie tijdens follow-up, en hoe gegevens van invasieve elektrofysiologie geprogrammeerde studies (EPS) te integreren. Dit proefschrift beoogt de ARVC Risk Calculator te valideren en te verbeteren, zodat deze in de dagelijkse klinische praktijk kan worden gebruikt.

Dit proefschrift bestaat uit twee delen.

Deel I, bestaande uit de hoofdstukken 3 tot en met 5, richt zich op de validatie van de ARVC Risk Calculator. Hoofdstuk 3 en 4 beschrijven de eerste onafhankelijke ervaringen met het algoritme, en hoofdstuk 4 bevestigt dat het kan worden gebruikt bij patiënten met uitgebreide blootstelling aan lichaamsbeweging zonder dat er aanpassingen nodig zijn. De studie die het algoritme volledig valideert wordt besproken in hoofdstuk 5.

Deel II, bestaande uit de hoofdstukken 6 tot en met 9, behandelt de verbeteringen die in de ARVC Risk Calculator zijn aangebracht. In hoofdstukken 6 en 7 wordt uitgelegd hoe gegevens van meerdere tijdens de follow-up uitgevoerde hartonderzoeken in het algoritme kunnen worden geïntegreerd om de voorspellingen te updaten en de klinische bruikbaarheid ervan in de loop van de tijd te handhaven. Hoofdstuk 8 beschrijft hoe gegevens van invasieve elektrofysiologische onderzoeken in het algoritme kunnen worden geïntegreerd om de ICD-implantatie bij patiënten met een gemiddeld aritmierisico te verbeteren. Ten slotte wordt in hoofdstuk 9 verslag uitgebracht over de werking van het algoritme bij een specifieke subgroep van ARVC-patiënten met pathogene varianten in het Desmoplakine-gen, die unieke kenmerken hebben in vergelijking met andere ARVC-patiënten (namelijk een vroegtijdige ziektebetrokkenheid van de linkerkant van het hart).

Het laatste hoofdstuk 10 geeft een gedetailleerde samenvatting van dit proefschrift, waarbij de resultaten in perspectief worden geplaatst en worden besproken in het kader van het ARVC-onderzoek.

De belangrijkste bevindingen van het proefschrift kunnen als volgt worden samengevat:

1. De ARVC Risk Calculator is een betrouwbaar en gevalideerd algoritme gebleken voor het identificeren van patiënten die een ICD nodig hebben ten tijde van hun eerste ARVC-diagnose. Het is superieur aan andere risicostratificatie-instrumenten en vereist geen aanpassingen voor blootstelling aan inspanning. Het is klaar voor gebruik in de dagelijkse klinische praktijk.
2. Invasieve EPS kan helpen bij de risicostratificatie bij patiënten met ARVC. Wij hebben een versie van de ARVC Risico Calculator ontwikkeld die EPS-integratie mogelijk maakt. Het gebruik van EPS levert de nuttigste informatie op wanneer deze wordt uitgevoerd bij patiënten met een gemiddeld risico op VA's.
3. Door regelmatig cardiale onderzoeken te verrichten kunnen de risicoschattingen van de ARVC Risk Calculator worden geüpdatet, waardoor de follow-up op lange termijn wordt verbeterd.
4. Genotype is een belangrijke factor bij het bepalen van het risico van ritmestoornissen bij patiënten met ARVC. De ARVC Risk Calculator is effectief voor patiënten met klassieke, rechtszijdige ARVC, maar niet voor patiënten met ARVC veroorzaakt door genetische varianten van Desmoplakine.
5. Om patiënten met ARVC gepersonaliseerde zorg te kunnen bieden, is een gen-specifieke benadering van de risicostratificatie nodig. Sterke internationale samenwerking zal van cruciaal belang zijn voor het bereiken van het doel plotselinge hartdood bij jonge, ogenschijnlijk gezonde personen te voorkomen en tegelijkertijd het gebruik van ICD's tot een minimum te beperken.

CURRICULUM VITAE

Alessio Gasperetti was born on November 21st, 1993, in Milan, Italy.

He completed his senior year rotation at the Heart Rhythm Center of the IRCCS Centro Cardiologico Monzino in Milan, Italy. In 2018, he graduated summa cum laude from the Università degli Studi di Milano after defending his medical school thesis on Arrhythmic Cardiomyopathy, under the supervision of Profs. Dello Russo, Casella, and Tondo. He received an unrestricted license to practice medicine independently in Italy in 2019.

In 2021, Alessio started working as a Ph.D. student at UMC under the mentorship of Dr. Anneline te Riel and Dr. Peter van Tintelen, while attending an ARVC Research Fellowship at the Johns Hopkins University in Baltimore, USA, under the guidance of Dr. Hugh Calkins and Dr. Cindy James.

He completed his Ph.D. thesis in 2023 and was subsequently admitted to the Johns Hopkins Osler Medicine Training Program for a six-year combined residency and fellowship training program in Internal Medicine and Cardiology.

LIST OF PUBLICATIONS

PUBLISHED AND INDEXED CONTRIBUTIONS AT MAY 1st 2023 – 110 (From most recent)

110. Avran A, Zuffi A, Gobbi C, **Gasperetti A**, et al; *Gender-differences in percutaneous coronary intervention for chronic total occlusions from the ERCTO study* - **Catheter Cardiovasc Interv** *2023, Apr*

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DANKWOORD

The scientific process can feel like building a never-ending tower, where each brick is added by countless predecessors. In this final section, I want to express my gratitude to all the incredible individuals who have been part of the build-up of my very personal scientific journey across Europe and the USA.

Il primo ringraziamento è in italiano, ed è per i miei genitori. Probabilmente non hanno ancora capito esattamente cosa sia un dottorato di ricerca, perché io lo stia facendo in Olanda, quando finisca di specializzarmi e in che paese del mondo mi specializzerò. Non mi è però mai venuto a mancare il loro supporto né la loro pazienza durante questi anni. Con la speranza che tutto il viaggiare a cui li ho costretti li conservi in salute, non posso che ringraziarli con tutto il cuore per la loro presenza;

As far as family is involved, ***Aaron*** and ***Deb***, as well as ***Ella*** and ***Rainie*** (the ***"Kaplan's"***) should also be included. I am truly blessed of being a member of the extended Kaplan tribe. The majority of my success and my ability to stay in the USA is clearly indebted to your unwavering generosity. Your support has been the anchor that kept me grounded through the multiple storms of these past years. I solemnly promise never to try squeezing any more limes in your house.

Moving forward, I wish to extend my thanks to the extensive list of mentors, advisors, comrades, and colleagues who have joined me on this remarkable journey.

To ***Cindy James***, who originally planted the crazy idea of pursuing a Ph.D in the Netherlands. I am **not** a geneticist by training and I will never be, but your guidance has equipped me with enough knowledge to hold my own in scientific discussions without embarrassing myself (or so I hope!). I am eternally grateful for your supervision and patience during these adventurous three years and I look forward to the many years to come. Just a reminder, I will not put gene names in italics in any scientific paper unless explicitly reminded to do so!

To ***Peter van Tintelen*** and ***Anneline te Riele***, thank you for warmly accepting me into your mentorship, even with an ocean to set us apart. Through you, I have got to learn the wonderful (although sometimes alien) Dutch mindset. I am confident that our collaboration will continue to thrive in the years to come, as we embark on new endeavors together.

To ***Richard Carrick***, who has been an exceptional colleague, friend, chaperone, and unwavering supporter. Even after more than two years, I am still amazed by your eclectic collection of shirts and your adventurous choices when it comes to "flatbread" toppings. Thank you for your friendship and collaboration. Rest assured, I will make it my mission to properly educate you on the art of true beers and appropriate pizza toppings.

To ***Steven Muller***, you have been the epitome of a phenomenal Paranymph. You have single-handedly helped me navigate the intricacies of the stern Dutch bureaucracy, despite my chronic disorganization. This achievement is certainly worth celebrating and boasting about on your resume, alongside your impressive JACC paper. I believe this is just the beginning of a long and fruitful collaboration between us.

A special acknowledgment goes to ***Hugh Calkins***, an exquisite mentor and the perfect boss. It was through your guidance and support that I found my way to Hopkins three years ago and recently became a part of the Osler family. I mention this not only to express my gratitude but also to let others know who they can hold responsible for my persistent presence at JHU;

To ***Hari Tandri***, thank you for all the electrifying madness, the VT ablation studies, the memorable moments of allegedly escaped pigs in the Ross building, and the coffee breaks that fueled our conversations throughout the day. Your support has been invaluable and you almost convinced me to move to Nashville. I wish you the best of luck in your adventure at Vanderbilt and hope we will stay in touch for a long time;

To ***Brittney Murray***, the official core-lab and go-getter Ohio queen of the Johns Hopkins ARVC team. For all the support, the afternoon bitching, the margaritas, the on-call texting, and the patience shown when I sent you messy genetic data in the wrong format, I am forever in your debt;

To ***Crystal Tichnell***, the guardian angel ant behind-the-scenes coordinator of the ARVC team. Your timely updates, dad jokes, and relentless efforts to ensure everything runs smoothly have been invaluable. From the bottom of my heart, thank you for your dedication and support.

To ***Firat Duru*** and ***Ardan Saguner*** (peppeeepeee). Your mentorship during my Swiss ARVC fellowship played a pivotal role in my career and served as the catalyst for this thesis. I hold dear the memories of my time in Zurich, and I am grateful for your guidance and friendship. Thank you both for your invaluable support.

A ***Marco Schiavone***, compagno di mille avventure (scientifiche e non) e di almeno altrettante chiamate zoom notturne, anche se non so se mai leggerà questa tesi di dottorato siccome si occupa di una malattia "che non esiste e che vi siete tutti inventati". E' per me un privilegio unico il poter lavorare a così stretto contatto con un amico vero. Ora e sempre Schiavonismo e Rivoluzione;

A ***Paolo Compagnucci*** e ***Chiara Cappelletto***, compagni di trincea che con me condividono la sofferenza della raccolta dati per i molti e sempre nuovi progetti che scaturiscono;

A ***Sarah Costa***, lamantino inadatto alla vita approdato nelle spiagge di Zurigo. Per le telefonate in panico, le canzoni di Coez, l'allergia agli abbracci, e i biscotti. Trovati un ragazzo per favore.

A ***Michela Casella*** e ***Antonio Dello Russo***, perché l'inizio di questo percorso di ricerca non sarebbe stato possibile senza la loro guida;

Al ***Prof. Tondo*** e al ***Prof. Bartorelli***, per il supporto costante e la guida nel mio tentativo (sembrerebbe riuscito) di attraversare l'oceano;

A ***Giovanni Forleo***, per il costante supporto e l'amicizia maturata durante un folle quinquennio all'Ospedale Sacco. Oggi e sempre il mio Segretario AIAC;

A ***Luigi Di Biase*** e ***Pasquale Santangeli***, vero metro di paragone umano e professionale per tutti quelli che come me sognano l' EP American Dream . Non posso che essere grato per l'enorme supporto di entrambi che è stato, e spero continuerà a essere, fin qui determinante nel mio percorso;

Lastly, I would like to extend my gratitude to the individuals who have been an indispensable part of my personal life over the past three years. Without their presence and support, I might not have maintained even a sliver of sanity. Their influence has been immeasurable, and I am deeply thankful to each and every one of them.

Al mio gruppo di umanisti italiani con cui ho vissuto in “ufficio interno” a Brody. A ***Marc Flores***, per il calcio, le cene al CVP, le discussioni sugli stati nazione, le canzoni in dialetto, e le battute che fanno ridere solo noi. A ***Giacomo Loi***, per gli sguardi di sottecchi, per i cani rabidi, il pescato del giorno, e per i manierismi in aula studio. A ***Cristina***, che ringrazio anche se questa tesi viene difesa dopo il 31 di Maggio. Per il sarcasmo e la palestra. Per la possibilità di essere stato vero con te, sempre e fino alla fine;

To ***Emily Gerry***, who was the first to convince me to give staying in this country a chance. May your unwavering dedication to billing never wear you down;

To ***Neha Shah*** and ***Luciana Dinis*** (aka the Dingus), for the wonderful friendship we shared, the countless memories of the 2832 St. Paul Lifestyle, the lively Limoncello nights, and the Sunday brunches;

To ***Emily Graham***, for the continuous teasing ,the controversial Fridays, and your true Tennessee soul. I sincerely appreciate you and wish you the utmost success in your future career endeavors.

To all the other exceptional individuals who have accompanied me on this odyssey. Whether it was sharing laughter, brainstorming ideas, or enduring late-night experiments together, your presence and support have been invaluable.