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Rationale and study design of the MINERVA study: Multicentre Investigation of Novel Electrocardiogram Risk markers in Ventricular Arrhythmia prediction – UK multicentre collaboration

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Short Title: Rationale and Study Design MINERVA

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ABSTRACT

Objectives: To assess the ability of two new ECG markers (Regional Repolarisation Instability Index [R2I2] and Peak Electrical Restitution Slope [PERS]) in predicting sudden cardiac death (SCD) or ventricular arrhythmia (VA) events in patients with ischaemic cardiomyopathy undergoing implantation of an implantable cardioverter defibrillator for primary prevention indication. To further develop R2I2 and PERS as useful clinical risk stratification tools.

Methods: MINERVA is a prospective, open label, single blinded, multi-centre observational study to establish the efficacy of two ECG biomarkers in predicting ventricular arrhythmia risk. 440 participants with ischaemic cardiomyopathy undergoing routine first time ICD implantation for primary prevention indication are currently being recruited. An electrophysiological (EP) study is performed using a non-invasive programmed electrical stimulation protocol via the implanted device. All participants will undergo the EP study hence no randomisation is required. Participants will be followed up over a minimum of 18 months and up to 3 years. The first patient was recruited in August 2016 and the study will be completed at the final participant follow-up visit. The primary endpoint is ventricular fibrillation or sustained ventricular tachycardia ≥200bpm as recorded by the ICD. The secondary endpoint is sudden cardiac death. Analysis of the ECG data obtained during the EP study will be performed by the core lab where blinding of patient health status and endpoints will be maintained.

Conclusion: R2I2 and PERS were shown to be promising new ECG markers of SCD and VA in a single centre study but it is hoped that MINERVA will establish the evidence base for these to be developed into a useful clinical risk stratification tool.

ClinicalTrials.gov registration: NCT03022487

Keywords: sudden cardiac death, risk stratification, implantable cardioverter defibrillator

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study will provide strong validation of the performance of R2I2 and PERS as predictors of sudden cardiac death in a cohort of patients with ischaemic heart disease.
- R2I2 and PERS have previously shown strong positive predictive values suggesting that they may be of use in lower risk populations.
- This study is investigating a cohort of patients undergoing ICD implant; as such the primary endpoint of ventricular fibrillation or sustained ventricular tachycardia ≥200bpm is an imperfect surrogate for sudden cardiac death.
- A positive outcome would lead to a future study using R2I2 / PERS to determine ICD implantation with all cause mortality as the primary endpoint.

INTRODUCTION

Sudden cardiac death (SCD) is a major cause of mortality accounting for 4-5million deaths per year worldwide with coronary heart disease being the underlying aetiology in 80% of cases (1). Implantable cardioverter-defibrillator (ICD) technology has developed rapidly over the last four decades with the indications for implantation broadening in light of new evidence. Large randomised controlled trials have established their use in primary indications for preventing SCD and improving overall survival provided that patients are appropriately selected (2-6). Implant rates continue to rise with 238 per million population high energy devices implanted in England between 2015-2016 (7). Despite this, SCD remains an important cause of mortality because of the limitations of current risk assessment. The majority of deaths occur in those considered to be low-risk using current stratification criteria (8). Also, a substantial proportion of ICD recipients do not require therapy from their devices and ICDs carry a substantial morbidity with possible complications such as infection and inappropriate shocks (9). Imprecise ICD prescription can result in unnecessary cost with often suboptimal selection based on crude clinical parameters. SCD risk stratification therefore remains inadequate and a priority area for clinical research.

Action potential duration (APD) restitution describes an inherent property of the myocardium whereby the APD is dependent on the preceding diastolic interval (DI). The DI is the rest period between repolarisation and the next excitation. The relationship between APD and DI can be plotted on a restitution curve (10). The APD lengthens with longer DIs but at shorter DIs the APD restitution curve steepens such that small changes in DI cause a large change in APD.

APD restitution has been shown to be an important property in the genesis and maintenance of ventricular arrhythmia (VA). Ventricular fibrillation (VF) consists of multiple spiral re-entrant waves which have short lifetimes and require continual generation in order to persist and propagate. This is provided by wavebreak with electrical wavefront splitting into multiple wavelets (11).

APD restitution has been proposed to be associated with arrhythmogenesis by two different mechanisms. Firstly, the 'restitution hypothesis' states that the main determinant of wavebreak is the steepness of the restitution curve. When the gradient of the restitution curve is >1, small changes in DI can lead to a large change in APD and oscillations between APD and DI are magnified. Computer and experimental models have shown that steep curves promote instability and spiral wave breakup which in turn can lead to VF (12, 13). Secondly, heterogeneity of APD restitution in adjacent myocardium allows wavefronts to become dissonant, thereby providing a substrate for wavebreak and re-entry. APD restitution properties within the ventricle have been shown to display inter-ventricular, intra-ventricular, and transmural heterogeneity (14-16).

Two novel surface ECG markers have been developed based on the principles of these mechanisms (17). The Regional Restitution Instability Index (R2I2) reflects heterogeneous restitution behaviour in different regions of the heart quantified using inter-lead heterogeneity. It is derived from the difference of the mean standard deviation of the residuals from the mean gradient for each ECG lead across the range of diastolic intervals. In an initial retrospective study in patients with ischaemic

cardiomyopathy, R2I2 was shown to be associated with VA or death (18). It was subsequently evaluated in a prospective, blinded study of 60 patients where it successfully replicated the findings of the retrospective study. R2I2 was found to be significantly higher in those reaching the endpoint of VA/SCD compared with those that had not (1.11±0.09 vs 0.84±0.04, p=0.003). Using a predefined cut-off value from the retrospective study, patients with R2I2>1.03 had significantly higher rates of VA/SCD (p<0.0001) with a sensitivity, specificity, positive predictive value, and negative predictive value of 63%, 82%, 56%, and 86% respectively(19).

A second surface ECG marker that measures peak APD restitution gradient was also assessed within the same cohort of patients with ischaemic cardiomyopathy (19). Peak ECG Restitution Slope (PERS) was calculated as the mean of the peak restitution slopes across the 12 ECG leads. PERS was significantly higher in those experiencing VA/SCD than those that did not. Patients with PERS≥1.21 were shown to have an incidence ratio of 4.1 times than those with PERS<1.21. Combining both biomarkers, a R2I2≥1.03 and PERS ≥1.21 gave a sensitivity, specificity, positive predictive value and negative predictive value of 50%, 95%, 80%, and 84%. The relative risk of VA/SCD was 21.6 when compared to R2I2<1.03 and PERS<1.21 combined. The predictive utility of both markers for VA/SCD was independent of left ventricular ejection fraction and QRS duration. The two parameters were also independent of each other supporting the view that they reflect different arrhythmogenic mechanisms.

Although the findings from this study were statistically significant and replicated the findings of the initial retrospective study, a relatively small number of participants were recruited from a single UK centre. ICDs were implanted for both primary and

secondary indications in the prospective study with devices implanted for a secondary prevention indication accounting for the greatest proportion of VA/SCD endpoints. This potentially questions whether these ECG markers would retain significant predictive value in the primary prevention population given that implanting a device would need greater justification in such a patient. Both VA and SCD were combined as a single endpoint. Given that VA may be asymptomatic, self-terminating and not resulting in SCD, this combined endpoint would not adequately reflect the predictive risk of mortality.

The MINERVA (Multicentre Investigation of Novel Electrocardiogram Risk markers in Ventricular Arrhythmia prediction) study has been designed to address these issues aiming to further stratify VA and SCD risk for primary prevention in a multi-centre trial. The MINERVA Investigators group is part of the British Heart Rhythm Society Multicentre Trial Collaboration.

STUDY DESIGN

MINERVA is a prospective, open label, single-blinded, multi-centre observational study. The study is registered with ClinicalTrials.gov (NCT03022487) and National Institute of Health Research in the UK (Trial No. 31324). Patients with ischaemic cardiomyopathy undergoing a first time ICD or CRT-D implantation for primary prevention [according to UK NICE guidelines (20)] will undergo a standard 30-minute electrophysiological (EP) cardiac stimulation protocol performed at the end of the implant procedure. The EP test will be performed whilst recording a high resolution digital 12-lead ECG. There is no randomisation required as all study

participants will receive EP study at baseline. Standard clinical follow up will take place through the ICD clinic as per local arrangements. Blinding will be maintained at the core lab where the ECG analysis will be performed. The core lab will not have details of patient health status. The results derived from the EP study data will be correlated with event rates to establish the relationship of the ECG markers in predicting VA risk. The study is event-driven with a sample size of 440 patients. Recruitment for this study has begun with the first patient having been recruited in August 2016. The minimum follow-up period should be 18 months and a maximum of 3 years.

Patient and Public Involvement

The science underpinning this study and this research study were presented to patients and the public 17/11/2015.

OBJECTIVES

The primary objective is to investigate whether R2I2 and PERS are significantly higher in ischaemic cardiomyopathy patients reaching the endpoint of VA and/or SCD than those who do not during the follow up period. The secondary objectives are to assess whether significantly more endpoints are reached in patients with any of the following:

- R2I2≥1.03 in comparison to R2I2<1.03
- PERS≥1.21 in comparison to PERS <1.21
- both R2I2≥1.03 and PERS ≥1.21 in comparison to both R2I2<1.03 and PERS<1.21

The primary endpoint is ventricular fibrillation or sustained ventricular tachycardia at a rate greater than 200 beats per minute as recorded by the ICD. The secondary endpoint is SCD. The endpoints will be adjudicated by the Endpoint validation committee.

PATIENTS

The intended population for this study are patients with ischaemic cardiomyopathy attending for a de novo ICD implantation (including CRT-D) for primary prevention indications based on current National Institute for Health and Care Excellence (NICE) guidelines (Table 1)(20). Patients at 15 UK centres (Appendix 1) who meet the inclusion and exclusion criteria, as detailed below, will be considered for the study.

Inclusion Criteria

To be included in the study, patients must be aged over 18 years of age, able to give informed consent for participation, and able to comply with the study requirements. Female patients of childbearing potential must be willing to ensure the use of effective contraception by themselves or their partner during the course of the study. All patients must have a diagnosis of ischaemic cardiomyopathy, on stable medication (as defined as no more than a 100% increase or 50% decrease in current regular medication for at least four weeks prior to study entry), and be attending for a de novo, primary prevention ICD implantation based on NICE technology appraisal (TA314). They must be able to read and understand English and allow their GP and consultant,

if appropriate, to be notified of participation in the study. Lastly, they must be able to and agree to attend follow up at the study site until the closure of the study.

Exclusion criteria

Patients are excluded from this study if they are within 28 days of an acute coronary syndrome or cardiac surgery, scheduled for elective surgery or any procedure requiring general anaesthesia, are pregnant, lactating or planning a pregnancy during the course of the study, have significant renal disease (requiring renal replacement therapy and/or eGFR<15), severe liver disease (end stage or the presence of liver cirrhosis), are participating in another research study involving an investigational product in the last 12 weeks, are undergoing any interventional research, have contraindications for an EP study including haemodynamic instability, severe valvular pathology as defined by the British Society of Echocardiography (BSE) guidelines, have symptomatic coronary artery disease, or had a stroke within the last 12 months. Participants will be excluded if they have a significant disease or disorder which, in the opinion of the investigator, may put the participant at risk because of participation in the study, or may influence the result of the study, or affect the participant's ability to participate in the study. At the time of ICD implantation the following exclusions from the study apply: the right ventricular lead is not apically positioned, if it is adjudged by the implanting physician that the patient would require a VT therapy zone ≤200 beats per minute or if there is any ventricular bradycardia pacing indication.

Patient randomisation

No randomisation is required as all study participants will receive the EP study at baseline.

STUDY PLAN

The pathway for the study is shown in Figure 1. The minimum follow-up period is 18 months. The study opened 08/July/2016 and will close 30/06/2021, patients have been recruited by 15 different centres in the United Kingdom. Recruitment has been substantially prolonged because of the covid-19 epidemic.

Screening and Eligibility Assessment

Only patients already destined for implantation of an ICD will be approached to consider participation in the study. They may be identified from either an inpatient or outpatient referral process. Once identified, the research team at each site will confirm the suitability of individual patients by reviewing their medical history and notes. Eligibility will be confirmed from the assessment of basic demographics, medical history, concomitant medication, recent ECGs, cardiac function and blood tests. After informing the care team, the research team will then approach eligible patients either in person, when they are visiting the hospital for routine outpatient appointments, whilst they are inpatients awaiting the procedure itself, or via telephone conversation to enquire if they would like to be considered for the study. The patient information leaflet and informed consent form will be given or sent to the patient for full consideration. They will be allowed a minimum of 24 hours to consider the

information. Written informed consent will be obtained either at a subsequent visit or on the day of the implantation of the ICD.

Baseline assessment

Following recruitment, demographic information, medical history, concomitant medication, basic blood chemistry, ECG parameters, and left ventricular ejection fraction (as assessed by either echocardiography or magnetic resonance imaging) will be confirmed and documented as baseline data.

Non-invasive EP study via ICD

At ICD implantation, the deliverable therapy zones will be programmed at rates ≥200 beats per minute with a monitor-only zone from 150 beats per minute. The parameters will otherwise be programmed according to the manufacturer specific guidelines as per the 2015 Consensus Statement on Optimal ICD Programming and Testing (21).

The EP study is performed using a single extrastimulus protocol as per the standard non-invasive physiological stimulation (NIPS) function of ICDs from all manufacturers and will be performed through the device immediately post-implantation.

Recording of the digital 12-lead ECG during the EP study protocol will be performed using a portable high-resolution 1kHz sampling digital ECG recorder (Norav 1200-HR [Norav Medical, Weisbaden, Germany]) and the company software and hardware (USB dongle) included with the PC interface. The risks of the non-invasive EP study

are small (1.3% risk of arrhythmia) (22). Unlike a standard VT stimulation study, the aim is not to provoke ventricular arrhythmia. The objective is simply to obtain a range of values from which to derive R2I2 and PERS. The non-invasive EP procedure would add no more than 30 minutes to the standard care of the ICD implant.

Programmed stimulation will be delivered at the RV apex via the ICD using the manufacturer-specific programmer. Rectangular pulses will be delivered with a pulse duration of 2 ms and output at three times the diastolic threshold. The drive train (S1) length is 10 beats followed by one extrastimulus (S2). For valid data the final two S1 of the drive train and S2 must successfully capture in succession (Figure 2), or else the drive train would be repeated.

The EP study protocol consists of two stages which are both repeated. For Stage 1, a drive cycle length (DCL) of 600 ms is followed by a single S2 at 500 ms. Drive trains are to be repeated with S1-S2 coupling interval decremented by 20 ms to 300 ms and then by 10 ms to the effective refractory period (ERP). If breakthrough beats are seen consistently in the drive train, the DCL should be reduced to 500 ms with the S1-S2 interval starting at 460 ms.

For Stage 2, the DCL is 400 ms with the initial coupling interval at 360 ms, decremented by 20 ms to 300 ms and then by 10 ms to ERP.

Subsequent assessment and follow up

Standard clinical follow up will take place through the ICD clinic as per local arrangements. The initial appointment usually takes place 4-6 weeks post-implantation and subsequently every 6 months. During the appointments, an ICD interrogation will be performed with the report exported as part of routine clinical care by a cardiac physiologist or a suitably trained investigator. Some centres may utilise home monitoring. During the appointments, the research team will recheck eligibility, reconfirm willingness to participate, assess and record the presence of arrhythmia-related endpoints, record current medications, and report any adverse events. Information not requiring device interrogation can be obtained through telephone interviews. At preset time intervals (12 and 18 months), the local PI will assess the presence of endpoints from patient notes and record the exact time to the first endpoint if present.

Analysis of R2I2 and PERS

The ECGs recorded during the EP study will be exported at 16-bit resolution for analysis. The digital ECG data will be transferred to the core lab for analysis using custom software written in MATLAB (Mathworks, Natick, USA). All data analysis and calculation of the R2I2 and PERS values will be performed by an investigator blinded to the clinical endpoints. A step-wise linear-fitting method, which is a standard approach, will be used to construct restitution curves using surface ECG surrogates for APD and DI (Q-T_{peak} interval and T_{peak}-Q interval) as described in previous works (Figure 3) (10, 19). For each lead of the surface ECG, the Q-T_{peak} is plotted against the T_{peak}-Q. Gradients are fitted for each 40ms overlapping least square linear segment. For each lead, in each 40 ms segment, the difference of the gradient

from the mean gradient is calculated. The mean of the standard deviations is taken as the R2I2. The mean of the peak restitution curve slope is calculated to be the PERS value.

Statistical considerations

Digital ECG data obtained from the EP study will be securely transferred to the Core Lab for prospective analysis and calculation (blinded to clinical outcome) of R2I2 and PERS. Once 12- and 18- month endpoint assessment have been made for each participant, the study groups will be divided into those reaching endpoint and those not reaching endpoint. Based on existing data, R2I2 data are expected to be parametric and PERS data non-parametric. Parametric data will be expressed as mean (±SEM) and analysed using a Student's t-test; non-parametric data as median [interquartile range] and analysed using the Mann-Whitney U test. Proportions will be analysed using a two-sided Fisher's Exact test. Previous work (18, 19) has found that an R2I2 value of 1.03 and PERS value of 1.21 provide the best 'cut-off' values to partition patients into 'high' and 'low' risk groups, from which Kaplan-Meier survival curves can be drawn for patient subgroups partitioned by this R2I2 cut-off and for patient subgroups partitioned by combinations of R2I2 and PERS cut-offs; comparison of cumulative endpoints will again be based on logarithmic transformations and survival will be recorded as time to first endpoint or the end of follow up.

Sample size

The sample size was informed by a two-sample t-test power calculation using the Satterthwaite approximation for unequal variances and using the R2I2 data from a previous study (R2I2 in VA/SCD group compared to non-VA/SCD, mean±SD 1.11±0.36 vs. 0.84±0.27)(19). To achieve over 90% power at a 5% significance level requires a minimum of 22 patients reaching endpoint. The endpoint rate is estimated to be 5% based on MADIT-RIT study data (23). Hence, to achieve 22 endpoints during 12 months of follow up, a sample size of 440 participants will be required. A p-value of <0.05 will be considered to be significant.

Ethics and monitoring

The Steering Committee consists of the study PI's (Appendix 1) who are responsible for the clinical and scientific conduct of the study and the publication of the results. In addition, the Steering Committee will review Adverse Events (AE) and Serious Adverse Events (SAE). The Research Coordinator will prepare the endpoints for adjudication by the Endpoints Committee who will not have access to blinded data (Appendix 2).

The study design and research protocol were approved by the Research Ethics Committees Northern Ireland (Reference No. 16/NI/0069) and Health Research Authority (IRAS reference 186618, EDGE ID 51707) with informed consent being obtained from the subjects. The study is being conducted in accordance with UK laws, Good Clinical Practice, and the Declaration of Helsinki 2002.

DISCUSSION

Left ventricular ejection fraction (LVEF) is the current predominantly used, least-worst tool for ICD risk stratification. The reliance on this marker leads to a large number of patients who, on the basis of LVEF, are considered low risk but go on to have SCD. This is whilst a substantial proportion of patients receiving ICDs do not make use of them; this results in considerable, unnecessary cost and morbidity. In the MADIT-II and SCD-HeFT trials, in which reduced LV function was the main marker of risk, only 10% of patients received appropriate ICD shock therapy per year during the 4-year follow up period(4, 5).

Basic science research on electrical restitution has been extended into translational work that has led to the development of two novel risk markers of sudden cardiac death. R2I2 and PERS represent a technology using familiar ECG recording equipment and can be performed with minimal specialist training. R2I2 and PERS are both independent of left ventricular ejection fraction in their association with VA/SCD occurrence. This raises the possibility that R2I2/PERS will retain sufficient positive predictive value in a lower risk population and it is anticipated that it will enable reclassification of patients who are currently stratified as low or medium risk to be identified to receive ICDs to prevent SCD.

CONCLUSION

This multi-centre study is required to establish a strong evidence base for the efficacy of R2I2 and PERS in those currently felt to be at high risk of SCD. If this study confirms the predictive efficacy in this population, then it may be extrapolated to other patient groups in future studies.

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DISCLOSURES

The University of Leicester has applied for a patent for the R2I2 and PERS ECG markers on behalf of WBN and GAN.

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AM's salary is supported by Abbott (formerly St Jude Medical). The other investigators do not have conflicts of interest relating to this study.

CONTRIBUTORSHIP STATEMENT

This study was conceived by G Andre Ng and Will B Nicolson. All the authors: G Andre Ng, Amar Mistry, Michelle Newton, Fernando Schlindwein, Craig Barr, Matthew GD Bates, Jane Caldwell, Moloy Das, Mohsin Farooq, Neil Herring, Pier Lambiase, Faizel Osman, Manav Sohal, Andrew Staniforth, Muzahir Tayebjee, David Tomlinson, Zachary Whinnett, Arthur Yue, Will B Nicolson, were responsible for the design of the study and the acquisition of the data. All the authors were involved in the drafting of this work, final approval of the manuscript and are accountable for the work.

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LEGENDS

Figure 1. Study flowchart.

Figure 2. Example of captured stimulus. For valid data the final two S1 of the drive train and S2 must successfully capture in succession, or else the drive train should be repeated.

Figure 3: Derivation of R2I2 and PERS (A) Stimulation protocol demonstrating the fiducial points of $T_{peak}Q$ and QT_{peak} (blue) which are required to plot on the restitution curve (B) gradients are fitted for each 40ms overlapping least square linear segment. The mean of the standard deviations of gradient differences from the mean gradient is taken as the R2I2. The mean of the peak restitution curve slope is calculated to be the PERS value (Reproduced with permission from Nicolson 2014)(19).

Table 1. Treatment options with ICD or CRT for people with heart failure who have left ventricular dysfunction with an LVEF of 35% or less (according to NYHA class, QRS duration, LBBB, left bundle branch block, NYHA, New York Heart Association. Adapted from NICE technology appraisals [TA314] (2014)(20).

QRS interval	NYHA Class			
	I	II	III	IV
<120 ms	ICD if there is a high risk of SCD			ICD/CRT not clinically indicated
120-149 ms without LBBB	ICD	ICD	ICD	CRT-P
120-149 ms with LBBB	ICD	CRT-D	CRT-P or CRT-D	CRT-P
≥150 ms with / without LBBB	CRT-D	CRT-D	CRT-P or CRT-D	CRT-P

Table 1.

Table 1. Treatment options with ICD or CRT for people with heart failure who have left ventricular dysfunction with an LVEF of 35% or less (according to NYHA class, QRS duration, LBBB, left bundle branch block, NYHA, New York Heart Association. Adapted from NICE technology appraisals [TA314] (2014)(20).

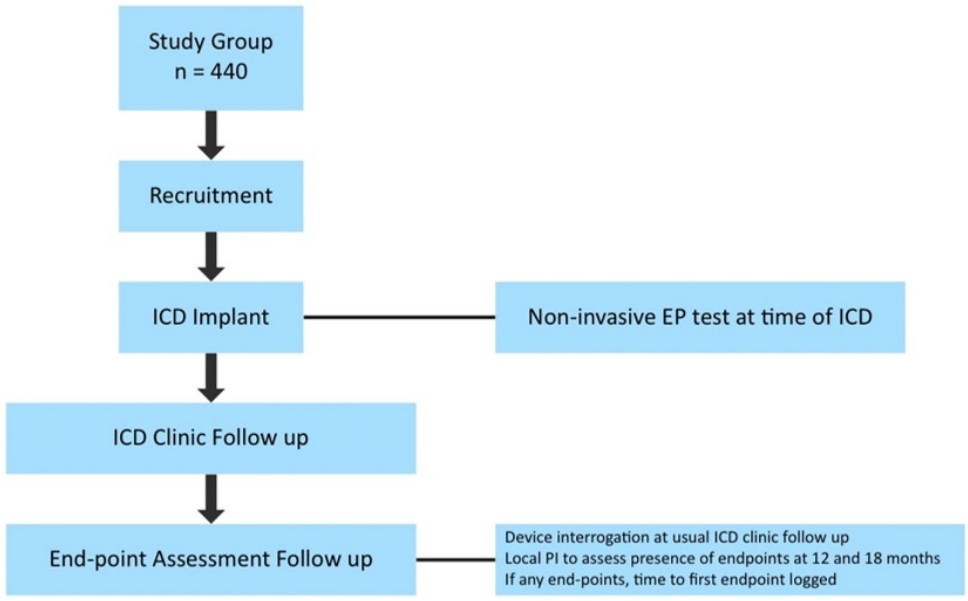


Figure 1. Study flowchart.

225x154mm (96 x 96 DPI)

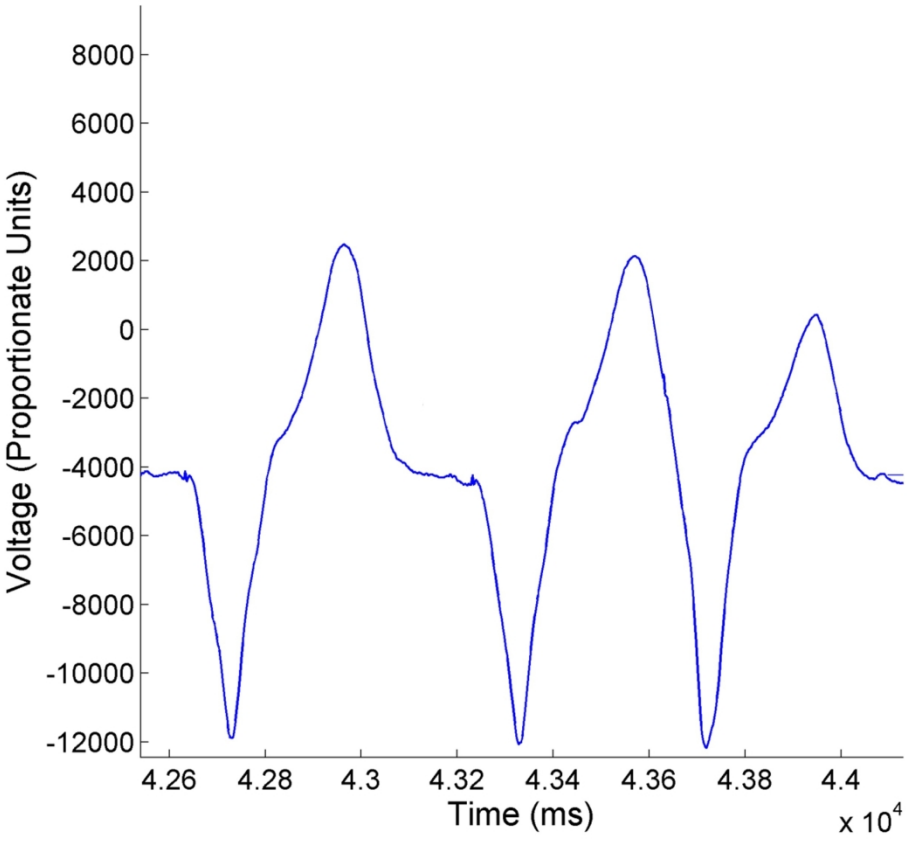
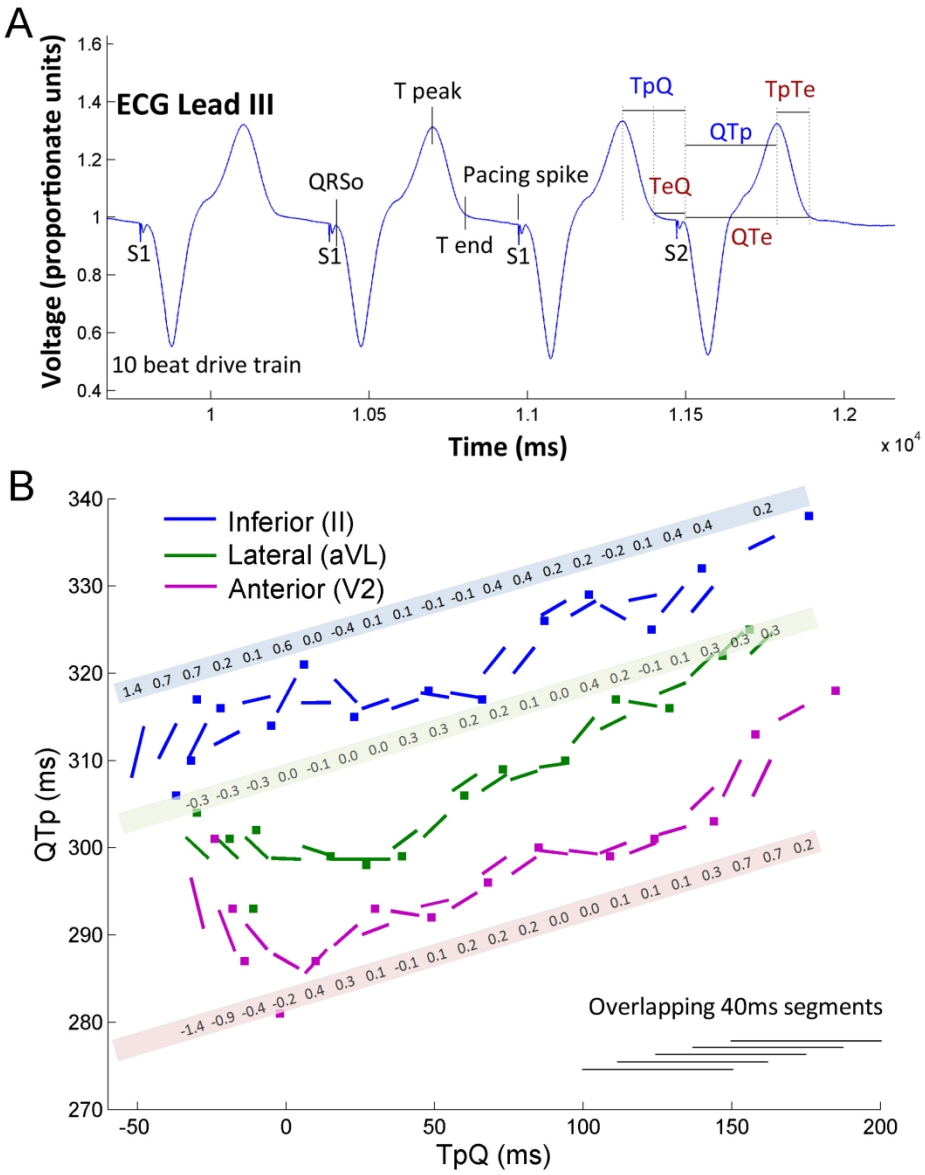


Figure 2. Example of captured stimulus. For valid data the final two S1 of the drive train and S2 must successfully capture in succession, or else the drive train should be repeated.

206x190mm (300 x 300 DPI)



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Appendix 1 – Chief Investigator and Principal Investigators

Chief Investigator:

Prof GA Ng (University of Leicester)

Principal Investigators:

Prof GA Ng (University Hospitals of Leicester NHS Trust),

Dr C Barr (Dudley Group NHS Foundation Trust),

Dr M Bates (South Tees Hospitals NHS Trust)

Dr J Caldwell (Hull and East Yorkshire NHS Trust),

Dr M Das (The Newcastle Upon Tyne Hospitals NHS Foundation Trust),

Dr M Farooq (Kettering General Hospital NHS Foundation Trust)

Prof N Herring (Oxford University Hospitals NHS Foundation Trust),

Prof P Lambiase (University College London Hospitals NHS Foundation Trust),

Prof F Osman (University Hospitals Coventry and Warwickshire NHS Trust),

Dr M Sohal (St. George's University Hospitals NHS Foundation Trust),

Dr A Staniforth (Nottingham University Hospitals NHS Trust),

Dr M Tayebjee (Leeds Teaching Hospitals NHS Trust),

Dr D Tomlinson (Plymouth Hospitals NHS Trust),

Dr Z Whinnett (Imperial College Healthcare NHS Trust),

Dr A Yue (University Hospital Southampton NHS Foundation Trust)

Appendix 2 – Endpoint Committee

Chair of Endpoint Committee:

Prof F Leyva (Aston University, Birmingham)

Members of Endpoint Committee:

Dr Shajil Chalil (Blackpool Hospital)

Dr Rachel Myles (University of Glasgow)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Described in abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Described in abstract
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported See introduction page 3.
Objectives	3	State specific objectives, including any prespecified hypotheses See objectives section page 8
Methods		
Study design	4	Present key elements of study design early in the paper Study design section page 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Study plan page 11.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants See screening and eligibility page 11.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable See baseline assessment and description of tests pages 10-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group See baseline assessment and description of tests pages 10-13
Bias	9	Describe any efforts to address potential sources of bias See study plan page 11.
Study size	10	Explain how the study size was arrived at See sample size page 15.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why See statistical considerations page 15.
Statistical methods	12	See statistical considerations page 15.

	(*a*) Describe all statistical methods, including those used to control for confounding
	(*b*) Describe any methods used to examine subgroups and interactions
	(*c*) Explain how missing data were addressed
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy See statistical considerations page 15.
	(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Recruitment ongoing
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Recruitment ongoing.
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures Recruitment ongoing.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Recruitment ongoing.
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Recruitment ongoing.
Discussion		
Key results	18	Summarise key results with reference to study objectives Recruitment ongoing.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Recruitment ongoing.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Recruitment ongoing.
Generalisability	21	Discuss the generalisability (external validity) of the study results Recruitment ongoing.
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based See Sources of funding page 18.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Rationale and study design of the MINERVA study: Multicentre Investigation of Novel Electrocardiogram Risk markers in Ventricular Arrhythmia prediction – UK multicentre collaboration

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine, Diagnostics
Keywords:	Ischaemic heart disease < CARDIOLOGY, Pacing & electrophysiology < CARDIOLOGY, Heart failure < CARDIOLOGY

Rationale and study design of the MINERVA study: Multicentre Investigation of Novel Electrocardiogram Risk markers in Ventricular Arrhythmia prediction – UK multicentre collaboration

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Short Title: Rationale and Study Design MINERVA

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ABSTRACT

Introduction: The purpose of this study is to assess the ability of two new ECG markers (Regional Repolarisation Instability Index [R2I2] and Peak Electrical Restitution Slope [PERS]) to predict sudden cardiac death (SCD) or ventricular arrhythmia (VA) events in patients with ischaemic cardiomyopathy undergoing implantation of an implantable cardioverter defibrillator for primary prevention indication.

Methods and analysis: MINERVA is a prospective, open label, single blinded, multi-centre observational study to establish the efficacy of two ECG biomarkers in predicting ventricular arrhythmia risk. 440 participants with ischaemic cardiomyopathy undergoing routine first time ICD implantation for primary prevention indication are currently being recruited. An electrophysiological (EP) study is performed using a non-invasive programmed electrical stimulation protocol via the implanted device. All participants will undergo the EP study hence no randomisation is required. Participants will be followed up over a minimum of 18 months and up to 3 years. The first patient was recruited in August 2016 and the study will be completed at the final participant follow-up visit. The primary endpoint is ventricular fibrillation or sustained ventricular tachycardia ≥200bpm as recorded by the ICD. The secondary endpoint is sudden cardiac death. Analysis of the ECG data obtained during the EP study will be performed by the core lab where blinding of patient health status and endpoints will be maintained.

Ethics and dissemination: Ethical approval has been granted by Research Ethics Committees Northern Ireland (Reference No. 16/NI/0069). The results will inform the design of a definitive RCT. Dissemination will include peer reviewed journal articles reporting the qualitative and quantitative results, as well as presentations at conferences and lay summaries.

ClinicalTrials.gov registration: NCT03022487

Keywords: sudden cardiac death, risk stratification, implantable cardioverter defibrillator

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study will provide strong validation of the performance of R2I2 and PERS as predictors of sudden cardiac death in a cohort of patients with ischaemic heart disease.
- R2I2 and PERS have previously shown strong positive predictive values suggesting that they may be of use in lower risk populations.
- This study is investigating a cohort of patients undergoing ICD implant; as such the primary endpoint of ventricular fibrillation or sustained ventricular tachycardia ≥200bpm is an imperfect surrogate for sudden cardiac death.
- A positive outcome would lead to a future study using R2I2 / PERS to determine ICD implantation with all cause mortality as the primary endpoint.

INTRODUCTION

Sudden cardiac death (SCD) is a major cause of mortality accounting for 4-5million deaths per year worldwide with coronary heart disease being the underlying aetiology in 80% of cases (1). Implantable cardioverter-defibrillator (ICD) technology has developed rapidly over the last four decades with the indications for implantation broadening in light of new evidence. Large randomised controlled trials have established their use in primary indications for preventing SCD and improving overall survival provided that patients are appropriately selected (2-6). Implant rates continue to rise with 238 per million population high energy devices implanted in England between 2015-2016 (7). Despite this, SCD remains an important cause of mortality because of the limitations of current risk assessment. The majority of deaths occur in those considered to be low-risk using current stratification criteria (8). Also, a substantial proportion of ICD recipients do not require therapy from their devices and ICDs carry a substantial morbidity with possible complications such as infection and inappropriate shocks (9). Imprecise ICD prescription can result in unnecessary cost with often suboptimal selection based on crude clinical parameters. SCD risk stratification therefore remains inadequate and a priority area for clinical research.

Action potential duration (APD) restitution describes an inherent property of the myocardium whereby the APD is dependent on the preceding diastolic interval (DI). The DI is the rest period between repolarisation and the next excitation. The relationship between APD and DI can be plotted on a restitution curve (10). The APD lengthens with longer DIs but at shorter DIs the APD restitution curve steepens such that small changes in DI cause a large change in APD.

APD restitution has been shown to be an important property in the genesis and maintenance of ventricular arrhythmia (VA). Ventricular fibrillation (VF) consists of multiple spiral re-entrant waves which have short lifetimes and require continual generation in order to persist and propagate. This is provided by wavebreak with electrical wavefront splitting into multiple wavelets (11).

APD restitution has been proposed to be associated with arrhythmogenesis by two different mechanisms. Firstly, the 'restitution hypothesis' states that the main determinant of wavebreak is the steepness of the restitution curve. When the gradient of the restitution curve is >1, small changes in DI can lead to a large change in APD and oscillations between APD and DI are magnified. Computer and experimental models have shown that steep curves promote instability and spiral wave breakup which in turn can lead to VF (12, 13). Secondly, heterogeneity of APD restitution in adjacent myocardium allows wavefronts to become dissonant, thereby providing a substrate for wavebreak and re-entry. APD restitution properties within the ventricle have been shown to display inter-ventricular, intra-ventricular, and transmural heterogeneity (14-16).

Two novel surface ECG markers have been developed based on the principles of these mechanisms (17). The Regional Restitution Instability Index (R2I2) reflects heterogeneous restitution behaviour in different regions of the heart quantified using inter-lead heterogeneity. It is derived from the difference of the mean standard deviation of the residuals from the mean gradient for each ECG lead across the range of diastolic intervals. In an initial retrospective study in patients with ischaemic

cardiomyopathy, R2I2 was shown to be associated with VA or death (18). It was subsequently evaluated in a prospective, blinded study of 60 patients where it successfully replicated the findings of the retrospective study. R2I2 was found to be significantly higher in those reaching the endpoint of VA/SCD compared with those that had not (1.11±0.09 vs 0.84±0.04, p=0.003). Using a predefined cut-off value from the retrospective study, patients with R2I2>1.03 had significantly higher rates of VA/SCD (p<0.0001) with a sensitivity, specificity, positive predictive value, and negative predictive value of 63%, 82%, 56%, and 86% respectively(19).

A second surface ECG marker that measures peak APD restitution gradient was also assessed within the same cohort of patients with ischaemic cardiomyopathy (19). Peak ECG Restitution Slope (PERS) was calculated as the mean of the peak restitution slopes across the 12 ECG leads. PERS was significantly higher in those experiencing VA/SCD than those that did not. Patients with PERS≥1.21 were shown to have an incidence ratio of 4.1 times than those with PERS<1.21. Combining both biomarkers, a R2I2≥1.03 and PERS ≥1.21 gave a sensitivity, specificity, positive predictive value and negative predictive value of 50%, 95%, 80%, and 84%. The relative risk of VA/SCD was 21.6 when compared to R2I2<1.03 and PERS<1.21 combined. The predictive utility of both markers for VA/SCD was independent of left ventricular ejection fraction and QRS duration. The two parameters were also independent of each other supporting the view that they reflect different arrhythmogenic mechanisms.

Although the findings from this study were statistically significant and replicated the findings of the initial retrospective study, a relatively small number of participants were recruited from a single UK centre. ICDs were implanted for both primary and

secondary indications in the prospective study with devices implanted for a secondary prevention indication accounting for the greatest proportion of VA/SCD endpoints. This potentially questions whether these ECG markers would retain significant predictive value in the primary prevention population given that implanting a device would need greater justification in such a patient. Both VA and SCD were combined as a single endpoint. Given that VA may be asymptomatic, self-terminating and not resulting in SCD, this combined endpoint would not adequately reflect the predictive risk of mortality.

The MINERVA (Multicentre Investigation of Novel Electrocardiogram Risk markers in Ventricular Arrhythmia prediction) study has been designed to address these issues aiming to further stratify VA and SCD risk for primary prevention in a multi-centre trial. The MINERVA Investigators group is part of the British Heart Rhythm Society Multicentre Trial Collaboration.

STUDY DESIGN

MINERVA is a prospective, open label, single-blinded, multi-centre observational study. The study is registered with ClinicalTrials.gov (NCT03022487) and National Institute of Health Research in the UK (Trial No. 31324). Patients with ischaemic cardiomyopathy undergoing a first time ICD or CRT-D implantation for primary prevention [according to UK NICE guidelines (20)] will undergo a standard 30-minute electrophysiological (EP) cardiac stimulation protocol performed at the end of the implant procedure. The EP test will be performed whilst recording a high resolution digital 12-lead ECG. There is no randomisation required as all study

participants will receive EP study at baseline. Standard clinical follow up will take place through the ICD clinic as per local arrangements. Blinding will be maintained at the core lab where the ECG analysis will be performed. The core lab will not have details of patient health status. The results derived from the EP study data will be correlated with event rates to establish the relationship of the ECG markers in predicting VA risk. The study is event-driven with a sample size of 440 patients. Recruitment for this study has begun with the first patient having been recruited in August 2016. The minimum follow-up period should be 18 months and a maximum of 3 years.

Patient and Public Involvement

The science underpinning this study and this research study were presented to patients and the public 17/11/2015.

OBJECTIVES

The primary objective is to investigate whether R2I2 and PERS are significantly higher in ischaemic cardiomyopathy patients reaching the endpoint of VA and/or SCD than those who do not during the follow up period. The secondary objectives are to assess whether significantly more endpoints are reached in patients with any of the following:

- R2I2≥1.03 in comparison to R2I2<1.03
- PERS≥1.21 in comparison to PERS <1.21
- both R2I2≥1.03 and PERS ≥1.21 in comparison to both R2I2<1.03 and PERS<1.21

The primary endpoint is ventricular fibrillation or sustained ventricular tachycardia at a rate greater than 200 beats per minute as recorded by the ICD. The secondary endpoint is SCD. The endpoints will be adjudicated by the Endpoint validation committee.

PATIENTS

The intended population for this study are patients with ischaemic cardiomyopathy attending for a de novo ICD implantation (including CRT-D) for primary prevention indications based on current National Institute for Health and Care Excellence (NICE) guidelines (Table 1)(20). Patients at 15 UK centres (Appendix 1) who meet the inclusion and exclusion criteria, as detailed below, will be considered for the study.

Inclusion Criteria

To be included in the study, patients must be aged over 18 years of age, able to give informed consent for participation, and able to comply with the study requirements. Female patients of childbearing potential must be willing to ensure the use of effective contraception by themselves or their partner during the course of the study. All patients must have a diagnosis of ischaemic cardiomyopathy, on stable medication (as defined as no more than a 100% increase or 50% decrease in current regular medication for at least four weeks prior to study entry), and be attending for a de novo, primary prevention ICD implantation based on NICE technology appraisal (TA314). They must be able to read and understand English and allow their GP and consultant,

if appropriate, to be notified of participation in the study. Lastly, they must be able to and agree to attend follow up at the study site until the closure of the study.

Exclusion criteria

Patients are excluded from this study if they are within 28 days of an acute coronary syndrome or cardiac surgery, scheduled for elective surgery or any procedure requiring general anaesthesia, are pregnant, lactating or planning a pregnancy during the course of the study, have significant renal disease (requiring renal replacement therapy and/or eGFR<15), severe liver disease (end stage or the presence of liver cirrhosis), are participating in another research study involving an investigational product in the last 12 weeks, are undergoing any interventional research, have contraindications for an EP study including haemodynamic instability, severe valvular pathology as defined by the British Society of Echocardiography (BSE) guidelines, have symptomatic coronary artery disease, or had a stroke within the last 12 months. Participants will be excluded if they have a significant disease or disorder which, in the opinion of the investigator, may put the participant at risk because of participation in the study, or may influence the result of the study, or affect the participant's ability to participate in the study. At the time of ICD implantation the following exclusions from the study apply: the right ventricular lead is not apically positioned, if it is adjudged by the implanting physician that the patient would require a VT therapy zone ≤200 beats per minute or if there is any ventricular bradycardia pacing indication.

Patient randomisation

No randomisation is required as all study participants will receive the EP study at baseline.

STUDY PLAN

The pathway for the study is shown in Figure 1. The minimum follow-up period is 18 months. The study opened 08/July/2016 and will close 30/06/2021, patients have been recruited by 15 different centres in the United Kingdom. Recruitment has been substantially prolonged because of the covid-19 epidemic.

Screening and Eligibility Assessment

Only patients already destined for implantation of an ICD will be approached to consider participation in the study. They may be identified from either an inpatient or outpatient referral process. Once identified, the research team at each site will confirm the suitability of individual patients by reviewing their medical history and notes. Eligibility will be confirmed from the assessment of basic demographics, medical history, concomitant medication, recent ECGs, cardiac function and blood tests. After informing the care team, the research team will then approach eligible patients either in person, when they are visiting the hospital for routine outpatient appointments, whilst they are inpatients awaiting the procedure itself, or via telephone conversation to enquire if they would like to be considered for the study. The patient information leaflet and informed consent form will be given or sent to the patient for full consideration. They will be allowed a minimum of 24 hours to consider the

information. Written informed consent will be obtained either at a subsequent visit or on the day of the implantation of the ICD.

Baseline assessment

Following recruitment, demographic information, medical history, concomitant medication, basic blood chemistry, ECG parameters, and left ventricular ejection fraction (as assessed by either echocardiography or magnetic resonance imaging) will be confirmed and documented as baseline data.

Non-invasive EP study via ICD

At ICD implantation, the deliverable therapy zones will be programmed at rates ≥200 beats per minute with a monitor-only zone from 150 beats per minute. The parameters will otherwise be programmed according to the manufacturer specific guidelines as per the 2015 Consensus Statement on Optimal ICD Programming and Testing (21).

The EP study is performed using a single extrastimulus protocol as per the standard non-invasive physiological stimulation (NIPS) function of ICDs from all manufacturers and will be performed through the device immediately post-implantation.

Recording of the digital 12-lead ECG during the EP study protocol will be performed using a portable high-resolution 1kHz sampling digital ECG recorder (Norav 1200-HR [Norav Medical, Weisbaden, Germany]) and the company software and hardware (USB dongle) included with the PC interface. The risks of the non-invasive EP study

are small (1.3% risk of arrhythmia) (22). Unlike a standard VT stimulation study, the aim is not to provoke ventricular arrhythmia. The objective is simply to obtain a range of values from which to derive R2I2 and PERS. The non-invasive EP procedure would add no more than 30 minutes to the standard care of the ICD implant.

Programmed stimulation will be delivered at the RV apex via the ICD using the manufacturer-specific programmer. Rectangular pulses will be delivered with a pulse duration of 2 ms and output at three times the diastolic threshold. The drive train (S1) length is 10 beats followed by one extrastimulus (S2). For valid data the final two S1 of the drive train and S2 must successfully capture in succession (Figure 2), or else the drive train would be repeated.

The EP study protocol consists of two stages which are both repeated. For Stage 1, a drive cycle length (DCL) of 600 ms is followed by a single S2 at 500 ms. Drive trains are to be repeated with S1-S2 coupling interval decremented by 20 ms to 300 ms and then by 10 ms to the effective refractory period (ERP). If breakthrough beats are seen consistently in the drive train, the DCL should be reduced to 500 ms with the S1-S2 interval starting at 460 ms.

For Stage 2, the DCL is 400 ms with the initial coupling interval at 360 ms, decremented by 20 ms to 300 ms and then by 10 ms to ERP.

Subsequent assessment and follow up

Standard clinical follow up will take place through the ICD clinic as per local arrangements. The initial appointment usually takes place 4-6 weeks post-implantation and subsequently every 6 months. During the appointments, an ICD interrogation will be performed with the report exported as part of routine clinical care by a cardiac physiologist or a suitably trained investigator. Some centres may utilise home monitoring. During the appointments, the research team will recheck eligibility, reconfirm willingness to participate, assess and record the presence of arrhythmia-related endpoints, record current medications, and report any adverse events. Information not requiring device interrogation can be obtained through telephone interviews. At preset time intervals (12 and 18 months), the local PI will assess the presence of endpoints from patient notes and record the exact time to the first endpoint if present.

Analysis of R2I2 and PERS

The ECGs recorded during the EP study will be exported at 16-bit resolution for analysis. The digital ECG data will be transferred to the core lab for analysis using custom software written in MATLAB (Mathworks, Natick, USA). All data analysis and calculation of the R2I2 and PERS values will be performed by an investigator blinded to the clinical endpoints. A step-wise linear-fitting method, which is a standard approach, will be used to construct restitution curves using surface ECG surrogates for APD and DI (Q-T_{peak} interval and T_{peak}-Q interval) as described in previous works (Figure 3) (10, 19). For each lead of the surface ECG, the Q-T_{peak} is plotted against the T_{peak}-Q. Gradients are fitted for each 40ms overlapping least square linear segment. For each lead, in each 40 ms segment, the difference of the gradient

from the mean gradient is calculated. The mean of the standard deviations is taken as the R2I2. The mean of the peak restitution curve slope is calculated to be the PERS value.

Statistical considerations

Digital ECG data obtained from the EP study will be securely transferred to the Core Lab for prospective analysis and calculation (blinded to clinical outcome) of R2I2 and PERS. Once 12- and 18- month endpoint assessment have been made for each participant, the study groups will be divided into those reaching endpoint and those not reaching endpoint. Based on existing data, R2I2 data are expected to be parametric and PERS data non-parametric. Parametric data will be expressed as mean (±SEM) and analysed using a Student's t-test; non-parametric data as median [interquartile range] and analysed using the Mann-Whitney U test. Proportions will be analysed using a two-sided Fisher's Exact test. Previous work (18, 19) has found that an R2I2 value of 1.03 and PERS value of 1.21 provide the best 'cut-off' values to partition patients into 'high' and 'low' risk groups, from which Kaplan-Meier survival curves can be drawn for patient subgroups partitioned by this R2I2 cut-off and for patient subgroups partitioned by combinations of R2I2 and PERS cut-offs; comparison of cumulative endpoints will again be based on logarithmic transformations and survival will be recorded as time to first endpoint or the end of follow up.

Sample size

The sample size was informed by a two-sample t-test power calculation using the Satterthwaite approximation for unequal variances and using the R2I2 data from a previous study (R2I2 in VA/SCD group compared to non-VA/SCD, mean±SD 1.11±0.36 vs. 0.84±0.27)(19). To achieve over 90% power at a 5% significance level requires a minimum of 22 patients reaching endpoint. The endpoint rate is estimated to be 5% based on MADIT-RIT study data (23). Hence, to achieve 22 endpoints during 12 months of follow up, a sample size of 440 participants will be required. A p-value of <0.05 will be considered to be significant.

Ethics, dissemination and monitoring

The Steering Committee consists of the study PI's (Appendix 1) who are responsible for the clinical and scientific conduct of the study and the publication of the results. In addition, the Steering Committee will review Adverse Events (AE) and Serious Adverse Events (SAE). The Research Coordinator will prepare the endpoints for adjudication by the Endpoints Committee who will not have access to blinded data (Appendix 2). The results will inform the design of a definitive RCT. Dissemination will include peer reviewed journal articles reporting the qualitative and quantitative results, as well as presentations at conferences and lay summaries.

The study design and research protocol were approved by the Research Ethics Committees Northern Ireland (Reference No. 16/NI/0069) and Health Research Authority (IRAS reference 186618, EDGE ID 51707) with informed consent being

obtained from the subjects. The study is being conducted in accordance with UK laws, Good Clinical Practice, and the Declaration of Helsinki 2002.

DISCUSSION

Left ventricular ejection fraction (LVEF) is the current predominantly used, least-worst tool for ICD risk stratification. The reliance on this marker leads to a large number of patients who, on the basis of LVEF, are considered low risk but go on to have SCD. This is whilst a substantial proportion of patients receiving ICDs do not make use of them; this results in considerable, unnecessary cost and morbidity. In the MADIT-II and SCD-HeFT trials, in which reduced LV function was the main marker of risk, only 10% of patients received appropriate ICD shock therapy per year during the 4-year follow up period(4, 5).

Basic science research on electrical restitution has been extended into translational work that has led to the development of two novel risk markers of sudden cardiac death. R2I2 and PERS represent a technology using familiar ECG recording equipment and can be performed with minimal specialist training. R2I2 and PERS are both independent of left ventricular ejection fraction in their association with VA/SCD occurrence. This raises the possibility that R2I2/PERS will retain sufficient positive predictive value in a lower risk population and it is anticipated that it will enable reclassification of patients who are currently stratified as low or medium risk to be identified to receive ICDs to prevent SCD.

ACKNOWLEDGEMENTS

The development of R2I2 and PERS includes studies which are part of the research portfolio supported by the National Institute for Health Research Leicester Biomedical Research Centre.

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DATA AVAILABILITY STATEMENT

No additional data available

DISCLOSURES

The University of Leicester has applied for a patent for the R2I2 and PERS ECG markers on behalf of WBN and GAN.

GAN reports research fellow support from Boston Scientific Ltd and Abbott Ltd.

AM's salary is supported by Abbott (formerly St Jude Medical). The other investigators do not have conflicts of interest relating to this study.

CONTRIBUTORSHIP STATEMENT

This study was conceived by G Andre Ng and Will B Nicolson. All the authors: G Andre Ng, Amar Mistry, Michelle Newton, Fernando Schlindwein, Craig Barr, Matthew GD Bates, Jane Caldwell, Moloy Das, Mohsin Farooq, Neil Herring, Pier Lambiase, Faizel Osman, Manav Sohal, Andrew Staniforth, Muzahir Tayebjee, David Tomlinson, Zachary Whinnett, Arthur Yue, Will B Nicolson, were responsible for the

design of the study and the acquisition of the data. All the authors were involved in the drafting of this work, final approval of the manuscript and are accountable for the work.

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LEGENDS

Figure 1. Study flowchart.

Figure 2. Example of captured stimulus. For valid data the final two S1 of the drive train and S2 must successfully capture in succession, or else the drive train should be repeated.

Figure 3: Derivation of R2I2 and PERS (A) Stimulation protocol demonstrating the fiducial points of $T_{peak}Q$ and QT_{peak} (blue) which are required to plot on the restitution curve (B) gradients are fitted for each 40ms overlapping least square linear segment. The mean of the standard deviations of gradient differences from the mean gradient is taken as the R2I2. The mean of the peak restitution curve slope is calculated to be the PERS value (Reproduced with permission from Nicolson 2014)(19).

Table 1. Treatment options with ICD or CRT for people with heart failure who have left ventricular dysfunction with an LVEF of 35% or less (according to NYHA class, QRS duration, LBBB, left bundle branch block, NYHA, New York Heart Association. Adapted from NICE technology appraisals [TA314] (2014)(20).

QRS interval	NYHA Class			
	I	II	III	IV
<120 ms	ICD if there is a high risk of SCD			ICD/CRT not clinically indicated
120-149 ms without LBBB	ICD	ICD	ICD	CRT-P
120-149 ms with LBBB	ICD	CRT-D	CRT-P or CRT-D	CRT-P
≥150 ms with / without LBBB	CRT-D	CRT-D	CRT-P or CRT-D	CRT-P

Table 1.

Table 1. Treatment options with ICD or CRT for people with heart failure who have left ventricular dysfunction with an LVEF of 35% or less (according to NYHA class, QRS duration, LBBB, left bundle branch block, NYHA, New York Heart Association. Adapted from NICE technology appraisals [TA314] (2014)(20).

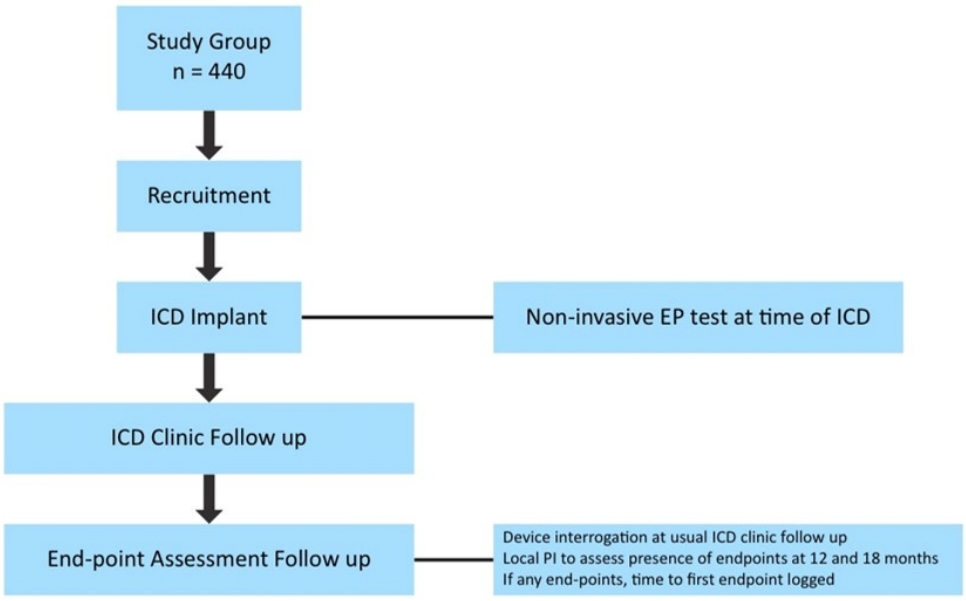

Figure 1. Study flowchart.

225x154mm (96 x 96 DPI)

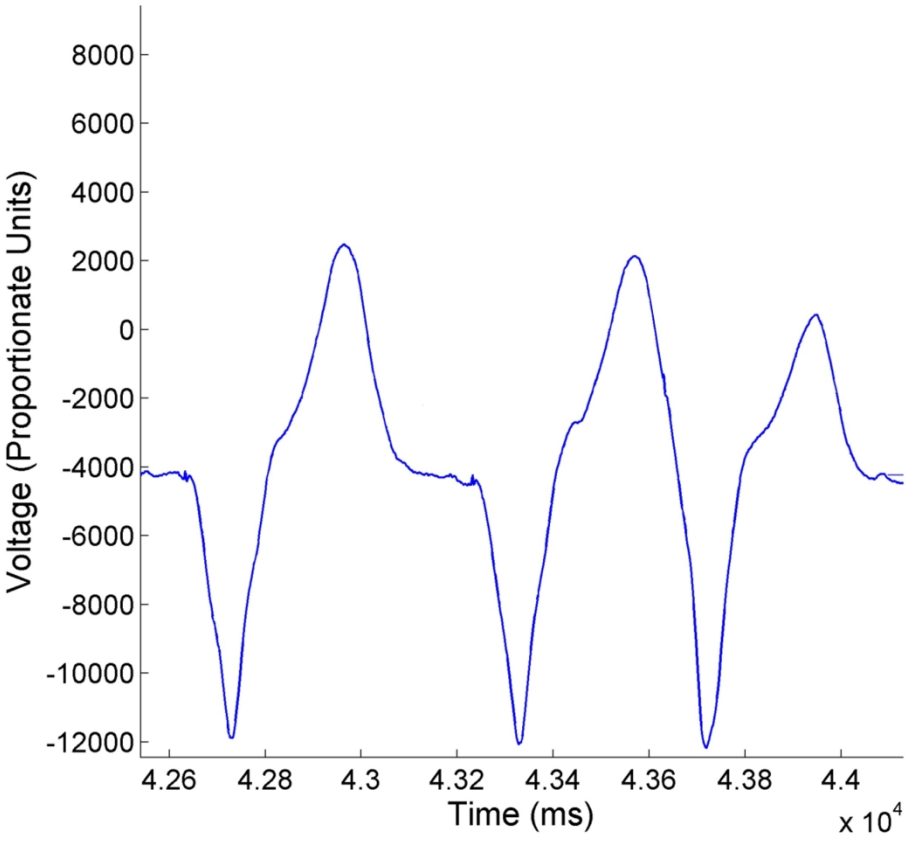

Figure 2. Example of captured stimulus. For valid data the final two S1 of the drive train and S2 must successfully capture in succession, or else the drive train should be repeated.

206x190mm (330 x 330 DPI)

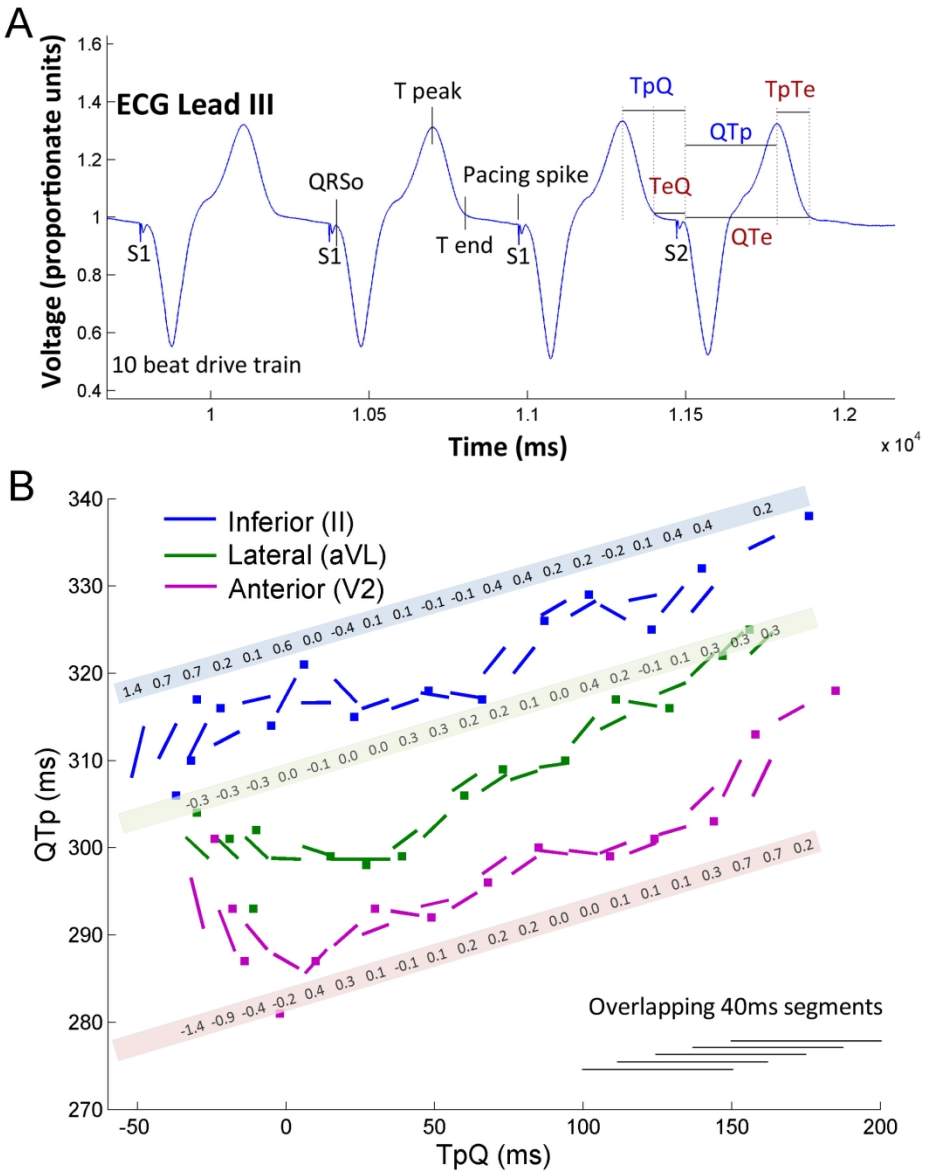

Figure 3: Derivation of R2I2 and PERS (A) Stimulation protocol demonstrating the fiducial points of TpeakQ and QTpeak (blue) which are required to plot on the restitution curve (B) gradients are fitted for each 40ms overlapping least square linear segment. The mean of the standard deviations of gradient differences from the mean gradient is taken as the R2I2. The mean of the peak restitution curve slope is calculated to be the PERS value (Reproduced with permission from Nicolson 2014)(19).

Appendix 1 – Chief Investigator and Principal Investigators

Chief Investigator:

Prof GA Ng (University of Leicester)

Principal Investigators:

Prof GA Ng (University Hospitals of Leicester NHS Trust),

Dr C Barr (Dudley Group NHS Foundation Trust),

Dr M Bates (South Tees Hospitals NHS Trust)

Dr J Caldwell (Hull and East Yorkshire NHS Trust),

Dr M Das (The Newcastle Upon Tyne Hospitals NHS Foundation Trust),

Dr M Farooq (Kettering General Hospital NHS Foundation Trust)

Prof N Herring (Oxford University Hospitals NHS Foundation Trust),

Prof P Lambiase (University College London Hospitals NHS Foundation Trust),

Prof F Osman (University Hospitals Coventry and Warwickshire NHS Trust),

Dr M Sohal (St. George's University Hospitals NHS Foundation Trust),

Dr A Staniforth (Nottingham University Hospitals NHS Trust),

Dr M Tayebjee (Leeds Teaching Hospitals NHS Trust),

Dr D Tomlinson (Plymouth Hospitals NHS Trust),

Dr Z Whinnett (Imperial College Healthcare NHS Trust),

Dr A Yue (University Hospital Southampton NHS Foundation Trust)

Appendix 2 – Endpoint Committee

Chair of Endpoint Committee:

Prof F Leyva (Aston University, Birmingham)

Members of Endpoint Committee:

Dr Shajil Chalil (Blackpool Hospital)

Dr Rachel Myles (University of Glasgow)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract Described in abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Described in abstract
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported See introduction page 3.
Objectives	3	State specific objectives, including any prespecified hypotheses See objectives section page 8
Methods		
Study design	4	Present key elements of study design early in the paper Study design section page 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Study plan page 11.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants See screening and eligibility page 11.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable See baseline assessment and description of tests pages 10-13
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group See baseline assessment and description of tests pages 10-13
Bias	9	Describe any efforts to address potential sources of bias See study plan page 11.
Study size	10	Explain how the study size was arrived at See sample size page 15.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why See statistical considerations page 15.
Statistical methods	12	See statistical considerations page 15.

	(*a*) Describe all statistical methods, including those used to control for confounding
	(*b*) Describe any methods used to examine subgroups and interactions
	(*c*) Explain how missing data were addressed
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy See statistical considerations page 15.
	(*e*) Describe any sensitivity analyses

Continued on next page

Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Recruitment ongoing
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Recruitment ongoing.
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures Recruitment ongoing.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Recruitment ongoing.
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Recruitment ongoing.
Discussion		
Key results	18	Summarise key results with reference to study objectives Recruitment ongoing.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Recruitment ongoing.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Recruitment ongoing.
Generalisability	21	Discuss the generalisability (external validity) of the study results Recruitment ongoing.
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based See Sources of funding page 18.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.