Rationale, objectives, and design of the EUTrigTreat clinical study: a prospective observational study for arrhythmia risk stratification and assessment of interrelationships among repolarization markers and genotype

Joachim Seegers[1], Marc A. Vos[2], Panagiota Flevari[3], Rik Willems[4], Christian Sohns[1], Dirk Vollmann[1], Lars Lüthje[1], Dimitrios T. Kremastinos[3], Vincent Floré[4], Mathias Meine[2], Anton Tuinenburg[2], Rachel C. Myles[5], Dirk Simon[6], Jürgen Brockmöller[7,9], Tim Friede[8,9], Gerd Hasenfuß[1,9], Stephan E. Lehnart[1,9], and Markus Zabel[1,9]* for the EUTrigTreat Clinical Study Investigators

[1]Department of Cardiology and Pneumology, University Medical Centre Göttingen, Georg-August-Universität, Göttingen, Germany; [2]Department of Physiology and Cardiology, Universitair Medisch Centrum Utrecht, Utrecht, The Netherlands; [3]2nd Department of Cardiology, Attikon Hospital, University of Athens, Athens, Greece; [4]Department of Cardiovascular Sciences, Division of Clinical Cardiology, University of Leuven, Leuven, Belgium; [5]BHF Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; [6]Institute for Clinical Research, University Medical Centre Göttingen, Göttingen, Germany; [7]Department of Clinical Pharmacology, University Medical Centre Göttingen, Göttingen, Germany; [8]Department of Medical Statistics, Centre for Informatics, Statistics and Epidemiology, University Medical Centre Göttingen, Göttingen, Germany; and [9]Heart Research Centre Göttingen, Göttingen, Germany

Aims	The EUTrigTreat clinical study has been designed as a prospective multicentre observational study and aims to (i) risk stratify patients with an implantable cardioverter defibrillator (ICD) for mortality and shock risk using multiple novel and established risk markers, (ii) explore a link between repolarization biomarkers and genetics of ion (Ca^{2+}, Na^+, K^+) metabolism, (iii) compare the results of invasive and non-invasive electrophysiological (EP) testing, (iv) assess changes of non-invasive risk stratification tests over time, and (v) associate arrythmogenomic risk through 19 candidate genes.
Methods and results	Patients with clinical ICD indication are eligible for the trial. Upon inclusion, patients will undergo non-invasive risk stratification, including beat-to-beat variability of repolarization (BVR), T-wave alternans, T-wave morphology variables, ambient arrhythmias from Holter, heart rate variability, and heart rate turbulence. Non-invasive or invasive programmed electrical stimulation will assess inducibility of ventricular arrhythmias, with the latter including recordings of monophasic action potentials and assessment of restitution properties. Established candidate genes are screened for variants. The primary endpoint is all-cause mortality, while one of the secondary endpoints is ICD shock risk. A mean follow-up of 3.3 years is anticipated. Non-invasive testing will be repeated annually during follow-up. It has been calculated that 700 patients are required to identify risk predictors of the primary endpoint, with a possible increase to 1000 patients based on interim risk analysis.
Conclusion	The EUTrigTreat clinical study aims to overcome current shortcomings in sudden cardiac death risk stratification and to answer several related research questions. The initial patient recruitment is expected to be completed in July 2012, and follow-up is expected to end in September 2014. Clinicaltrials.gov identifier: NCT01209494.

* Corresponding author. Tel: +49 551 39 10265; fax: +49 551 39 19127, Email: markus.zabel@med.uni-goettingen.de

Introduction

Sudden cardiac death (SCD) is one of the major challenges in modern healthcare, accounting for an estimated 600 000 deaths per year in Europe. In the majority of the cases, SCD is caused by ventricular tachycardia (VT) or ventricular fibrillation (VF).[1] Implantable cardioverter defibrillator (ICD) treatment has been established in both primary and secondary prevention of SCD.[2] Although a variety of non-invasive risk stratification techniques exist, effective risk stratification for the prevention of SCD in individual patients remains a major clinical challenge:[3,4] The indication for ICD therapy in primary prophylaxis of SCD is mainly based on reduced left ventricular ejection fraction (LVEF), <30–40%.[2] In patients with reduced LVEF in the Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT), the incidence of appropriate ICD therapy was found to be 31% over 5 years,[5] and appropriate shocks were correlated with increased mortality.[6] Recently, certain patient subgroups have been identified in whom death can occur before a life-saving shock for a malignant tachyarrhythmia.[7] On the other hand, the risk of inappropriate ICD shocks[8] is an important concern involving pain, anxiety, and reduced quality of life,[9] as well as possible life-threatening risks.[10]

As a consequence, at least some patients who receive an ICD in line with current guidelines, will do so without future clinical benefit, but with the cost of the device and the risk of complications. The EUTrigTreat clinical study—a multicentre observational risk stratification study—investigates whether comprehensive assessment of multiple electrophysiological (EP) risk stratification techniques that are independently associated with risk can improve risk stratification to improve patient selection for ICD therapy. In addition, invasive and non-invasive programmed stimulation have never been compared systematically, and hence, to address this, a non-randomized comparison of these two modes of EP study has been incorporated into the protocol.

Furthermore, we hypothesize that physiological or pathophysiological links exist between repolarization variables and genetic markers of cardiomyocyte ion metabolism. In particular, Ca^{2+}-, Na^{+}-, and K^{+}-dependent EP mechanisms are investigated through an array of prospectively screened candidate genes.

Finally, we aim to assess the dynamic time-dependent changes of the non-invasive risk stratifiers that we use again during follow-up as well as their prognostic implication.

Methods

Objectives and study design

The EUTrigTreat clinical ICD and arrhythmia risk investigation has been designed as a prospective observational cohort study, including comprehensive electrocardiogram (ECG)-based risk stratification, candidate gene analyses, and either non-invasive or invasive EP study. The study's objectives are (i) to risk stratify a large cohort of ICD patients for all-cause mortality and ICD shock risk and using a combination of:

T-wave morphology (TWM), signal-averaged ECG (SAECG), T-wave alternans (TWA), beat-to-beat variability of repolarization (BVR), 24-hour Holter monitoring for ambient arrhythmia, heart rate variability, and heart rate turbulence, as well as clinical characteristics including standard laboratory markers; (ii) to characterize the relationships between repolarization abnormalities identified by TWA, BVR, TWM, restitution of repolarization, and genetic candidate mechanisms of cardiac ion channel metabolism; (iii) to compare the results of invasive and non-invasive EP testing in the two subgroups of the study; (iv) to evaluate the dynamicity of time-dependent changes of BVR, TWM, TWA, LVEF, and Holter, and a possible change in their prognostic value over time; and (v) to evaluate 19 candidate genes with established or suspected EP phenotype linkage as risk mediators in ICD patients.

A total of 700 ICD patients will be recruited in five academic clinical centres in Europe until mid-2012. On recruitment of 700 patients, a sample size review will be carried out to determine whether additional recruitment of up to 1000 ICD patients is necessary. Subjects scheduled for *de novo* ICD implantation or generator replacement are eligible for invasive EP testing, whereas patients from the outpatient ICD clinic undergo non-invasive EP testing. Indications for ICD implantation are independent of study participation and follow current guidelines.[2] *Tables 1* and *2* summarize the patient inclusion and exclusion criteria. Patients with coronary artery disease, including those with a history of ST elevation myocardial infarction may be enrolled up to a limit of 60% of the total number of patients. Patients with atrial fibrillation as the underlying rhythm may be enrolled up to a limit of 20% of the total number of patients.

Endpoints

Primary endpoint

The primary study endpoint is all-cause mortality during follow-up until September 2014. The expected mean follow-up time is 3.3 years based on the enrolment of 700 patients, and 2.8 years for the enrolment of 1000 patients according to the pre-specified interim statistical analysis.

Secondary endpoints

Secondary endpoints of the study are:

Sudden cardiac death, cardiac death, and non-cardiac death: The cause of death will be adjudicated by a blinded endpoint committee and each death will be classified as one of the following: SCD, cardiac death, and non-cardiac death. Sudden cardiac death is defined as death due to any cardiac disease and occurrence within 1 h after the onset of symptoms. A cardiac death is defined as any death presumed to have occurred from a cardiac cause other than SCD. Non-cardiac deaths are all other deaths.

Appropriate and inappropriate ICD shocks: Implantable cardioverter defibrillator therapies will be adjudicated by the blinded endpoint committee and classified as appropriate or inappropriate. An ICD shock is classified as appropriate if delivered for a ventricular tachyarrhythmia in the VT or VF detection zone. Based on the episode data stored by the device, an appropriate ICD shock is classified as (i) primarily delivered in the VF zone, (ii) delivered as a backup to failed antitachycardia pacing (ATP) in the VT zone, or (iii) delivered after acceleration of a failed ATP into the VF zone.

Table 1 Inclusion criteria

Clinical indication (primary and secondary prevention of SCD) for ICD implantation, ICD generator exchange; or chronically implanted ICD

Age ≥ 18 years

Written informed consent

Negative pregnancy test in women of childbearing potential

No participation in other clinical trials within 1 month before and after enrolment into the study

ICD, implantable cardioverter defibrillator; SCD, sudden cardiac death.

Table 2 Exclusion criteria

For invasive and non-invasive EP groups

Unstable cardiac disease, such as decompensated heart failure (NYHA Class IV) or acute coronary syndrome or symptomatic arrhythmias

Percutaneous coronary intervention or coronary artery bypass graft surgery ≤3 months ago

RV pacing >20% of the time in single- and dual-chamber device patients presenting for generator exchange

For non-invasive EP group

ICDs unable to deliver programmed ventricular stimulation via programmer

Cardiac resynchronization therapy devices <6 months after implantation

EP, electrophysiological; ICD, implantable cardioverter defibrillator; NYHA, New York Heart Association; RV, right ventricular; SCD, sudden cardiac death.

Each appropriate shock will be considered as a secondary endpoint of the study.

Each inappropriate shock will be classified as caused either by oversensing of cardiac or non-cardiac electrical signals such as VT or VF, or by inappropriate classification of supraventricular tachycardia as VT by the device, as verified by interpretation of the stored electrograms.

Secondary endpoint combining all-cause mortality and appropriate ICD shocks: The combination of all-cause mortality and appropriate ICD shocks is defined as a composite secondary endpoint. The combination of appropriate and inappropriate ICD shocks, i.e. any ICD shocks, is defined as another secondary composite endpoint.

Implantable cardioverter defibrillator programming

The investigators have discussed a mandatory ICD programming; however, due to enrolment of patients in five different countries and with a wide variety of ICD indications, this has not been deemed possible. It is therefore mandatory to document all ICD settings including subsequent changes for the endpoints committee. In principle, it has been agreed to adhere to current programming guidelines as set forth by Spragg and Berger[11] as long as judged clinically useful for a respective patient.

Study protocol

The study will be conducted in accordance with the Declaration of Helsinki, the principles of the International Conference on Harmonization of Good Clinical Practice,[12] and with the approval of all local ethics committees. An outline of the study protocol is shown in *Figure 1*. After written informed consent, non-invasive ECG-based risk stratification, EP study, and laboratory samples will be completed. Echocardiography will be performed to measure LVEF. Cardiovascular functional capacity will be assessed using the New York Heart Association symptomatic class. Pulse rate, resting blood pressure, weight, and height will be measured. Cardiovascular drug treatment will be documented along with the presence or absence of the following co-morbidities: Obesity, renal disease, liver disease, anaemia, peripheral arterial disease, cerebral vascular disease, pulmonary disease, metabolic disease, hypertension, sleep apnea, tobacco use, hyperlipidaemia, and family history of inherited cardiac disease or sudden death. Standard laboratory parameters will be analysed, including creatinine, gamma-GT, high-sensitivity C-reactive protein, and N-terminal pro-brain natriuretic protein.

Non-invasive electrocardiographic risk stratification

Twelve-lead electrocardiogram for T-wave morphology
The 12-lead surface ECG will be recorded using a standard digital recording device. QRS duration, QT interval, QTc by the Bazett formula, and standard TWM will be measured. The latter will be automatically analysed as previously described,[13] and principal component analysis ratio, total cosine R-to-T, TWM dispersion, normalized T-wave loop area, T-wave loop dispersion, and relative T-wave residuum will be computed.

### Signal-averaged electrocardiogram for late potentials
Signal-averaged ECG will be recorded according to standard guidelines and using standard equipment. Positivity for late potentials will be determined, where at least two-third of positive criteria are present in accordance with standardized grading rules.[14]

T-wave alternans testing
Microvolt TWA testing will be performed using the Cambridge Heart system and heart rate elevation by means of exercise.[15] Exercise intensity is tailored to reach a target heart rate of 110–120 beats per minute (bpm). The TWA test will be analysed by the proprietary software immediately after the test. Test results will be graded in consensus of two blinded investigators with expertise in interpreting microvolt TWA results, according to the rules developed by Bloomfield *et al.*[15] The maximal TWA amplitude in the vector magnitude and ECG leads will also be recorded.

Holter electrocardiogram
A 24-h Holter monitoring will be performed using a standard clinical recording device. Data will be analysed for the number of premature ventricular complexes and the number of episodes and rate of non-sustained VT, normalized for a recording time of 24 h. Heart rate variability, including standard deviation of RR intervals (SDNN), root mean square of successive differences in RR intervals (RMSSD), and frequency domain parameters (low frequency/high frequency) will also be calculated.[16] Heart rate turbulence comprising turbulence onset and slope as well as acceleration and deceleration capacity will be computed.[17]
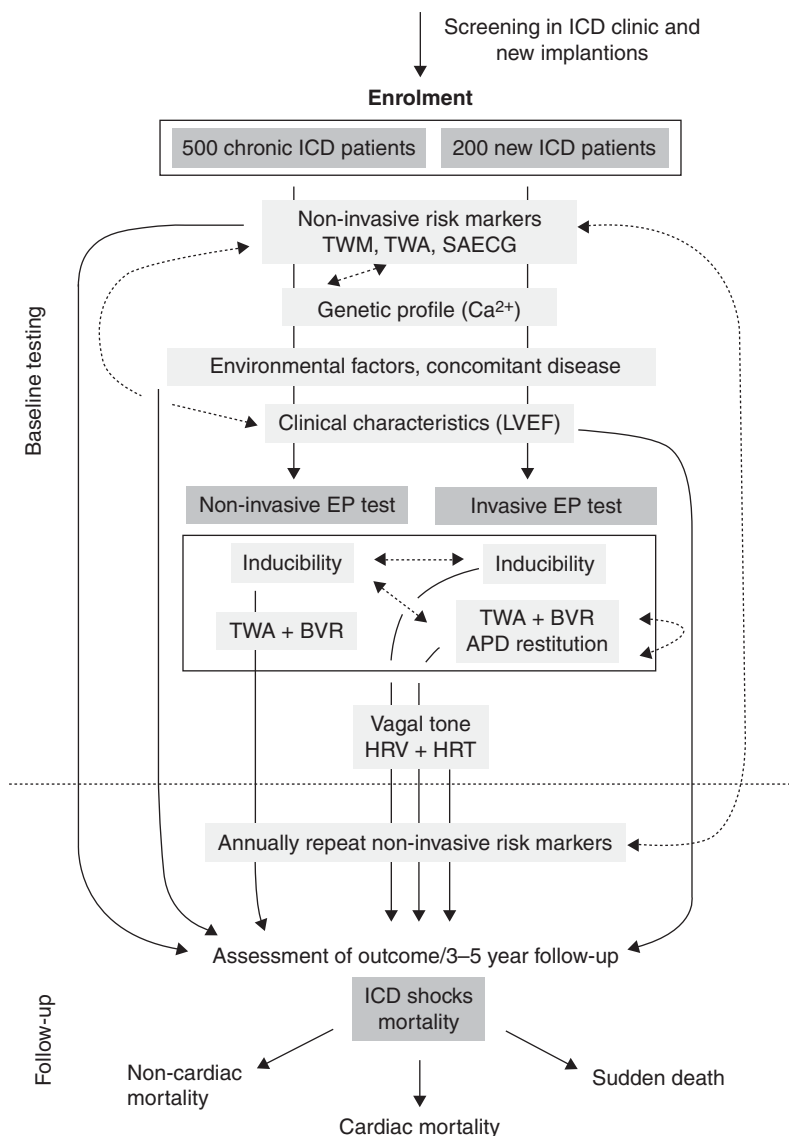
Figure 1 Study work flow. APD, action potential duration; BVR, beat-to-beat variability of repolarization [non-invasive part: 12-lead ECG (electrocardiogram), invasive part: 12-lead ECG and MAP (monophasic action potential)]; EP, electrophysiological; HRT, heart-rate turbulence; HRV, heart-rate variability; LVEF, left ventricular ejection fraction; ICD, implantable cardioverter defibrillator; SAECG, signal-averaged ECG; TWA, T-wave alternans; TWM, T-wave morphology.

All the described non-invasive risk stratifying ECG methods, except for SAECG, will be repeated at 6- and 12-month follow-up, and then annually until the end of the study in September 2014.

Electrophysiological study

Invasive electrophysiological study

In patients scheduled for new ICD implantations or undergoing ICD generator replacement, an invasive EP study will be performed. Standard diagnostic EP catheters will be placed in the high right atrium and in the His bundle position. A standard steerable Ag–AgCl quadripolar catheter will be positioned in the right ventricular (RV) apex and outflow tract to record and pace the monophasic action potential (MAP).[18] Inducibility of ventricular arrhythmias will be tested using an abbreviated Ann Arbor protocol[19] with four extrastimuli from two RV locations (RV apex and RV outflow

tract). For comparability with the patient cohort undergoing non-invasive EP testing, the protocol will also be conducted with three extrastimuli. The 12-lead ECG and MAPs will be recorded at various cycle lengths (1000, 857, 750, 667, 600, 545, 500, and 430 ms) during steady-state atrial and RV pacing for BVR analysis[20] as well as during atrially paced stepwise TWA.[21] Right ventricular pacing with a single extrastimulus, starting at 600 ms basic cycle length and decreasing towards refractoriness, will be performed for calculation and graphical depiction of the electrical restitution curve.[22] Furthermore, MAP signals will be analysed for action potential duration at 90% repolarization.[23]

Non-invasive electrophysiological study

In patients with a chronically implanted ICD device (>3 months), an EP study will be performed non-invasively using the ICD programmer.

The EP protocol is similar to the invasive EP study, except that MAP recordings will not be performed. Similar to the invasive EP study, inducibility of ventricular arrhythmias will be tested following an abbreviated Ann Arbor protocol with three extrastimuli from the RV electrode, as most of the ICD programmers cannot deliver four extrastimuli. Comparability with inducibility in invasively studied patients will be ensured by conducting invasive EP studies with both three and four extrastimuli. The 12-lead ECG for BVR and TWA analysis will be recorded at the above-mentioned cycle lengths during atrial and ventricular pacing wherever applicable, depending on the patient's ICD model and underlying rhythm. In patients receiving cardiac resynchronization therapy with underlying atrioventricular block, biventricular-paced TWA will be performed.[21] Beat-to-beat variability of repolarization will be repeated after 6 months and then once a year.

Candidate gene analysis

One rationale of the EUTrigTreat consortium is to characterize genetic arrhythmia modifiers in patients with different cardiomyopathies and to further differentiate arrhythmic substrates through a combined translational experimental approach. In this regard, the scientific focus is based on cardiac myocyte calcium and sodium metabolism while other candidate genes were included to allow for more comprehensive patient genotyping, for instance, also involving potassium channel variants as well as inherited cardiomyopathies.

Based on existing linkage and genome-wide association study (GWAS) data, we have identified 19 candidate genes of interest (*Table 3*). Inherited genetic variants in these 19 genes may increase the risk of ventricular arrhythmias and SCD in some patients. The candidate genes include different cation channels and their subunits, genes involved in intracellular calcium storage and homeostasis, sarcomeric, and cytoskeletal genes.[24–28] Beta-1-receptor polymorphisms were included because earlier studies have shown an as-yet controversial functional role of these polymorphisms in heart failure. Allelic variants of the HRC gene have been associated with an increased risk for malignant ventricular arrhythmias in 123 patients with idiopathic dilated cardiomyopathy.[26] The 19 candidate genes are characterized through select single-nucleotide polymorphism analysis and additional deep gene sequencing. Analyses of promoter sequences and exon–intron boundaries complement the genomic risk analysis. Based on recent data from whole genome massively parallel exome resequencing,[29] we expect to identify ~500 variants that may be associated with individual arrhythmogenic risk. The genetic data will be correlated both with non-invasive and invasive surrogate arrhythmogenic markers and physiological parameters. An important aim are association studies that address an objective of gene–environment interactions phenotypically manifested as traits of cardiac EP behaviour, for example TWA or electrical restitution. In addition, correlation of genomic variation with clinical outcomes is also of interest, but limited by overall sample size and therefore not available as GWAS strategy. However, in a secondary analysis, we will attempt to identify genes associated with the outcome variables. With an anticipated 500 variants, the predefined conservative estimate of the level of significance for genetic variables is 0.0001. In addition to the primary analysis, metabolic phenotypes including diabetes and obesity will be analysed in relation to the anticipated 500 genetic variants with minor allele frequencies of >3%. Furthermore, the genetic variants will be classified into six groups according to their genomic localization and likelihood of functionality, and these data will be associated with the primary and secondary outcomes. Our primary hypothesis in the analysis of all the genomic variants is the existence of a co-dominant mode of inheritance, i.e. the phenotype of heterozygous carriers is expected to lie between the phenotype of homozygous wild type and homozygous variant genotype for a given arrhythmia risk trait.

Committees

Clinical study steering panel

This panel will discuss the progress and timeline of the clinical study, and will supervise the study management. In cooperation with the centralized clinical research organization monitoring, this committee enforces adherence to good clinical practice and ethical standards. Clinical as well as biostatistical expertise will be provided on this panel. Because of the non-interventional nature of the present cohort study, no independent data safety monitoring board has been established for the study.

Endpoint committee

This committee is responsible for adjudicating mode of death and ICD shocks. For this purpose, database information, clinical narratives as well as original ICD episode printouts are made available for the committee meetings.

Statistics

Sample-size calculation

Three pivotal ICD studies involving ICD populations treated for primary and secondary prophylaxis[5,30,31] were considered in the sample-size calculation. In the AVID trial[30] of secondary ICD prophylaxis, a 2-year mortality of 18.4% and a 3-year mortality of 24.6%, respectively, were observed in the ICD group. A history of myocardial infarction was prevalent in 67% of 1016 patients. The DEFINITE study[31] enrolled 458 patients with non-ischaemic dilated cardiomyopathy, and a total of 229 patients were randomized to primary prevention ICD therapy. Their all-cause mortality over 4 years was 21%. Third, the SCD-HeFT trial[5] for primary ICD prophylaxis of SCD was conducted in 2521 patients with symptomatic heart failure and LV dysfunction. Of these, 52% exhibited ischaemic cardiomyopathy, while the remaining 48% had non-ischaemic cardiomyopathy. Among three treatment arms, 820 patients were randomized to ICD treatment. Their all-cause mortality was 28.9% after 5 years, with 35.9% for the ischaemic cardiomyopathy subgroup and 21.4% for the non-ischaemic cardiomyopathy subgroup. The MADIT-II trial[32] exclusively enrolled patients with ischaemic cardiomyopathy and was therefore not considered for sample-size calculations. Integrating the information from the three trials, all-cause mortality is observed to be higher in patients receiving ICD therapy for secondary prophylaxis of SCD, while patients

Table 3 Candidate genes involved in diseases at risk of sudden cardiac death

Channelopathies of sodium channels	*SCN5A*	*SCN1B*
Channelopathies of potassium channels	*KCNQ1*	*KCNH2*
Cellular calcium metabolism	*NCX1*	*HSP20*
	ANK2	*CASQ2*
	PLN	*HAX1*
	HRC	*RyR2*
	ASPH	
Cardiac cytoskeleton	*DMD, ANK2*	
Hypertrophic cardiomyopathy	Troponin (*TNNT2,TNNI3,TNNC1*)	
Adrenergic beta-1 receptor	*ADRB1*	

with ischaemic cardiomyopathy exhibit a higher mortality than those with non-ischaemic cardiomyopathy. Annual mortality ranges between 4.3% for non-ischaemic cardiomyopathy/primary prophylaxis in SCD-HeFT, 5.3% for non-ischaemic cardiomyopathy/primary prophylaxis in DEFINITE, 7.2% for ischaemic cardiomyopathy/ primary prophylaxis in SCD-HeFT, and 9.2% for ischaemic and non-ischaemic cardiomyopathy/secondary prophylaxis in AVID. In the EUTrigTreat clinical study, enrolment of a high proportion of patients with secondary prevention ICDs is expected. Furthermore, up to 60% of the patients with ischaemic cardiomyopathy and reduced LVEF are enrolled. We therefore anticipate a mortality of ≈7% per year, or 28% over a 4-year mean follow-up, for the purpose of sample-size calculation. A sample size of 632 patients then provides 80% power at a two-sided significance level of 5%, assuming 4-year mortalities of 23 and 33% in equally sized low- and high-risk groups, respectively, defined by a binary risk predictor (constituting a risk ratio of 1.65). Such predictors may be an ECG test, EP study result, or a genetic pattern, and might result from dichotomizing a continuous variable. Allowing for 10% withdrawals, we aim to recruit a total of 700 patients. Owing to improved pharmacological treatment and optimized revascularization since the conduct of the three pivotal ICD studies, the respective risk numbers derived may need to be adjusted towards significantly lower risk for the enrolment and observation period involved for the current study. Although these sample-size calculations are based on the best mortality estimates available from the literature, some uncertainty remains with regard to a potentially lower long-term mortality rate in the particular population enrolled. Therefore, the sample size will be recalculated after inclusion of the first 700 patients in June 2012, and might be raised up to a maximum total sample size of 1000 patients in the event of lower-than-expected event rates.[33,34] Enrolment will be stopped 6 months before the end of the study at the latest, providing a follow-up of ≥6 months in every patient. This sample size is expected to be sufficient for analyses involving secondary endpoints, ICD shocks, and appropriate shocks, for which 4-year probabilities of 64 and 50%, respectively, are assumed. Risk differences between equally sized risk groups of 11 percentage points for ICD shocks and 12 percentage points for appropriate shocks will lead to power values in excess of 80%. Importantly, the study is not powered for correlation of its genetic results with outcome. Therefore, correlations of genetic results with phenotype or phenotypic findings of the diagnostic methods are prioritized. A control group of patients without an ICD was not considered due to the overall very low risk of such patients and the fact that low-risk patient subgroups are expected from within the 'diverse-risk' ICD cohort.

Statistical analysis plan

In univariate analyses, the effects of dichotomized continuous variables and categorical variables will be described by Kaplan–Meier curves compared by log-rank tests. Correction for multiplicity of testing is planned by identification of correlations between the variables, i.e. those of autonomic tone. Cox regression analyses will be performed to quantify the predictive value of combinations of categorical variables and dichotomized continuous variables on the primary and secondary time-to-event endpoints (e.g. mortality or shocks). The independent predictive value of any variable will be determined. Useful combinations of independently predictive variables will be explored to establish risk scores for the prediction of mortality, ICD shocks, or any of the predefined primary or secondary endpoints. The significance of predictive genetic markers from high-dimensional data will be assessed in Cox regression models using appropriate approaches, such as boosting or permutation tests with potential confounders being included as covariates. Classification models will be validated using cross-validation.

Time line

The first patient was enrolled in January 2010; currently, 340 patients have been included. Recruitment of 700 patients is expected to be complete in July 2012, with a possible extension to 1000 patients scheduled until June 2014. The study ends in September 2014.

Conflicts of interest: none declared.

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