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Biomarkers for prediction of ventricular arrhythmias

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Abbreviations

ANP	Atrial Natriuretic Peptide
ATP	Antitachycardia pacing
BNP	B-type natriuretic peptide
BS	Brugada syndrome
CAD	Coronary artery disease
CDM	Dilated cardiomyopathy
CNP	C-type Natriuretic Peptide
CRP	C-reactive protein
cTn	Cardiac Troponin
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
CV	Coefficients of variation
CVD	Cardiovascular disease
DM	Diabetes mellitus
eGFR	Glomerular filtration rate
ESC	European Society of Cardiology guidelines
fQRS	Fragmentation of QRS
GDF-15	Growth differentiation factor 15
HF	Heart failure
HFmrEF	HF with mildly reduced EF
HFrEF	HF with reduced EF
HFpEF	HF with preserved EF
hs-cTn	Highly sensitive cardiac troponin
ICD	Implantable cardioverter defibrillator
IL-6	Interleukin 6
LVEF	Left ventricular ejection fraction
MEF	Mechano-electric feedback
MI	Myocardial infarction
NPs	Natriuretic peptides
NT-proBNP	N-terminal pro-B-type natriuretic peptide
NYHA	New York Heart Association
RAAS	Renin-angiotensin-aldosterone system
SCD	Sudden cardiac death
SNS	Sympathetic nervous system
TGF-β	Transforming growth factor-β
TnC	Troponin C
TOF	Tetralogy of Fallot
VA	Ventricular arrhythmia
VF	Ventricular fibrillation
VT	Ventricular tachycardia

Articles in the thesis

Paper I: **QRS fragmentation is associated with increased risk of ventricular arrhythmias in high-risk patients; Data from the SMASH 1 Study.**

Sourour, N., Riveland, E., Rømo, T., Næsgaard, P., Kjekshus, H., Larsen, A. I., Omland, T., Røsjø, H. & Myhre, P. L.

Ann Noninvasive Electrocardiol. 2022;27(5):e12985.

Paper II: **N-terminal pro-B-type natriuretic peptide for prediction of ventricular arrhythmias: data from the SMASH Study**

Sourour, N., Riveland, E., Næsgaard, P., Kjekshus, H., Larsen, A. I., Omland, T., Røsjø, H. & Myhre, P. L.

Accepted in Clinical Cardiology

Paper III: **The associations between biomarkers of myocardial injury and systemic inflammation and risk of incident ventricular arrhythmia; data from the SMASH 1 Study**

Sourour, N.*, Riveland, E.*, Næsgaard, P., Kjekshus, H., Larsen, A. I., Røsjø, H., Omland, T.** , & Myhre, P. L.**
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(Submitted manuscript, under review)

Thesis summary

Sudden cardiac death (SCD) is one of the leading causes of death in the world, and despite advances in cardiopulmonary resuscitation and post-resuscitation care, survival rates of cardiac arrests are poor. However, advances in the pharmacological treatment of HF and improvements in preventive measures and therapy of cardiovascular diseases (CVD) have led to a decline in CVD mortality and SCD worldwide. SCD is mainly caused by ventricular arrhythmia (VA), and implantable cardioverter defibrillator (ICD) has consistently been shown to be the most effective preventive measure for SCD and VA. However, guideline recommendation for ICD treatment poorly discriminates patients at risk.

SCD has a complex pathophysiology owing to heterogeneous underlying conditions making prediction challenging. Several biomarkers and electrocardiogram (ECG) parameters have been proposed to play a role in the risk prediction of VA. Fragmentation of the QRS complex (fQRS) in ECG has been demonstrated to reflect myocardial scarring, which may represent an arrhythmical trigger. Circulating concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) reflect cardiac stress, cardiac troponin T (cTnT) reflects cardiac injury, and inflammation is reflected by growth differentiation factor 15 (GDF-15), interleukin 6 (IL-6) and C-reactive protein (CRP). Elevated levels of these biomarkers have been demonstrated to reflect an increased risk of cardiovascular events (e.g., heart failure (HF), myocardial infarction (MI), stroke, atrial fibrillation, and CV death) in patients with and without CVD.

In this thesis we aimed to study the association between fQRS, NT-proBNP, cTnT, GDF-15, IL-6, and CRP each with the risk of device-detected VA, aiming to find better risk stratification tools to help predict potentially fatal VA in patients treated with ICD. We also studied their association with HF hospitalization and all-cause mortality. In **Paper I** we examined the association between variables derived from a standard 12-lead ECG and the risk of VA. In a pre-specified analysis plan, we decided to focus on fQRS, based on promising data from other clinical settings. In line with our hypothesis, we found that the presence of fQRS was associated with an increased risk of VA, and fQRS was the strongest risk predictor of all the ECG parameters investigated. This association was independent of established risk factors and was particularly strong among patients with a primary prevention ICD indication. fQRS was also associated with a combined endpoint of VA and mortality. In **Paper II** we wanted to assess whether the most frequently used biomarker in HF, NT-proBNP, was useful in predicting VA risk. We found that higher levels of NT-proBNP were associated with an increased risk of VA irrespective of established risk factors, such as left ventricular ejection fraction (LVEF). Interestingly, this association was stronger in patients with secondary, as compared to a primary, prevention ICD indication. As expected, higher NT-proBNP was also associated with a higher risk of incident HF hospitalization and all-cause mortality. Changes in NT-proBNP

concentrations from baseline to the follow-up visit were not associated with subsequent arrhythmical events but were associated with the risk of subsequent HF hospitalization and death. In **Paper III** we wanted to assess whether markers of myocardial injury and systemic inflammation were associated with VA risk. We found an association between higher cTnT levels and risk of VA, which was independent of established risk factors and present irrespective of ICD indication and ischemic vs. non-ischemic etiology. There were no associations between changes in cTnT concentration during the study and subsequent incidents of VA. In contrast, levels of GDF-15, IL-6, and CRP were not associated with VA risk. cTnT, GDF-15, IL-6, and CRP were all predictive of HF hospitalization and mortality, independently of established risk factors.

In conclusion, in this thesis we demonstrate that the presence of fQRS on ECG was associated with increased risk of VA and mortality in patients treated with ICD. Higher levels of the cardiac specific biomarkers NT-proBNP and cTnT, but not that of the inflammatory biomarkers GDF-15, IL-6 and CRP, were associated with an increased risk of VA. Concentrations of NT-proBNP, cTnT, GDF-15, IL-6 and CRP were all strong predictors of poor prognosis demonstrated by their ability to predict HF hospitalization and mortality. These data suggest that fQRS, NT-proBNP and cTnT may be useful in identifying patients at risk of VA and therefore may weigh in on decisions on the aggressiveness of antiarrhythmic treatment and perhaps whether to implant ICD in patients at risk of SCD.

Sammendrag på norsk

Plutselig hjertestans er en av hyppigste dødsårsakene, og til tross for fremskritt innen hjerte-lunge-redning og intensivbehandling etter resuscitering, er overlevelsesraten lav. Imidlertid har fremskritt innen farmakologisk behandling av hjertesvikt og forbedringer innen forebyggende tiltak og behandling av kardiovaskulære sykdommer ført til en nedgang i dødelighet av kardiovaskulære sykdommer og plutselig hjertedød. Plutselig hjertedød skyldes hovedsakelig ventrikulær arytmi (VA), og implanterbar kardioverter-defibrillator (ICD) har vist seg å være det mest effektive forebyggende tiltaket mot plutselig hjertedød og VA. Imidlertid er retningslinjene for ICD-behandling lite treffsikre når det gjelder å skille pasienter med høy og lav risiko for plutselig hjertedød.

Fordi ulike, til dels uavhengige, patofysiologiske mekanismer kan bidra til økt risiko for plutselig hjertedød, er risikovurdering utfordrende. Flere biomarkører og elektrokardiografiske (EKG) indekser/variabler har blitt foreslått å spille en rolle i vurderingen av fremtidig risiko for VA. Fragmentering av QRS-komplekset (fQRS) i EKG har blitt vist å gjenspeile arrdannelse i myokard, som kan være en utløsende faktor for arytmier. Sirkulerende biomarkører N-terminal pro-B-type natriuretisk peptid (NT-proBNP) som reflekterer hjertestress, hjertespesifikk troponin T (cTnT) som reflekterer hjerteskade og bettenelsesmarkører growth differentiation factor *15 (GDF-15), interleukin 6 (IL-6) og C-reaktivt protein (CRP) er alle sterke prognostiske markører. Høye sirkulerende nivåer av disse biomarkørene har vist seg å være forbundet med økt risiko for kardiovaskulære hendelser, som hjertesvikt, hjerteinfarkt, slag, atrieflimmer og kardiovaskulær død, både hos pasienter med og uten kardiovaskulær sykdom.*

I denne avhandlingen ønsket vi å studere fQRS, NT-proBNP, cTnT, GDF-15, IL-6 og CRP hver for seg, i assosiasjon med risiko for VA. Målet var å finne bedre verktøy for risikovurdering som kan hjelpe til med å forutsi tilfeller av VA som uten ICD behandling ville vært potensielt dødelige. Vi undersøkte også sammenhengen mellom disse markørene og sykehusinnleggelse grunnet hjertesvikt og total dødelighet. I ***artikkel I*** *undersøkte vi sammenhengen mellom EKG variabler fra standard 12-avlednings EKG og risikoen for VA. Vi valgte på forhånd å fokusere på fQRS, og teste hypotesen om fQRS er forbundet med økt risiko for VA. Vi bekreftet at tilstedeværelsen av fQRS var assosiert med økt risiko for VA. Denne assosiasjonen, uavhengig av etablerte risikofaktorer, var særlig sterk blant pasienter med ICD som primærprofylakse. fQRS var også assosiert med et kombinert endepunkt av VA og dødelighet. I* ***artikkel II*** *ønsket vi å vurdere om NT-proBNP, den mest brukte biomarkøren for hjertesvikt, var nyttig for å predikere risikoen for VA. Vi fant at høyere nivåer av NT-proBNP var assosiert med økt risiko for VA, uavhengig av etablerte risikofaktorer som venstre ventrikkels ejeksjonsfraksjon. Interessant nok var denne assosiasjonen sterkere hos pasienter med ICD som sekundærprofylakse enn hos de med primærprofylaktisk ICD. Som forventet var også NT-proBNP assosiert med risikoen for sykehusinnleggelse grunnet hjertesvikt og total dødelighet. Endringer i NT-proBNP-konsentrasjoner fra første til andre studiebesøk hadde ingen sammenheng med påfølgende episoder med arytmi, men var assosiert med risikoen for påfølgende sykehusinnleggelse grunnet hjertesvikt og død. I* ***artikkel III***

ønsket vi å teste hypotesene om at markører for hjertemuskelskade og systemisk betennelse var assosiert med risikoen for VA. Vi fant en sammenheng mellom høyere nivåer av cTnT og risikoen for VA, som var uavhengig av etablerte risikofaktorer og uavhengig av ICD-indikasjon og om pasienten hadde kjent koronarsykdom. Det var ingen sammenhenger mellom endringer i cTnT-konsentrasjoner i løpet av studien og påfølgende hendelser av VA. Vi fant ingen sammenheng mellom konsentrasjon av GDF-15, IL-6 og CRP og VA i løpet av observasjonstiden. Både cTnT, GDF-15, IL-6 og CRP var forbundet med risiko for sykehusinnleggelse grunnet hjertesvikt og dødelighet, uavhengig av etablerte risikofaktorer.

Konklusjonen er at tilstedeværelsen av fQRS i EKG er assosiert med økt risiko for VA og død hos pasienter som behandles med ICD. Høyere nivåer av de hjertespesifikke biomarkørene NT-proBNP og cTnT, men ikke de inflammatoriske biomarkørene GDF-15, IL-6 og CRP, er assosiert med økt risiko for VA. Konsentrasjoner av NT-proBNP, cTnT, GDF-15, IL-6 og CRP er alle sterkt forbundet med dårlig prognose, vist ved deres evne til å forutsi sykehusinnleggelse grunnet hjertesvikt og dødelighet. Våre data tyder på at at fQRS, NT-proBNP og cTnT kan være nyttige for å identifisere pasienter med økt risiko for VA, og kan dermed muligens påvirke indikasjonsstillingen for antiarytmisk behandling og ICD implantasjon hos pasienter med økt risiko for plutselig hjertedød.

1. General Introduction

1.1 Sudden cardiac death

Sudden cardiac death (SCD) is a major international public health challenge accounting for approximately 50% of cardiovascular (CV) deaths worldwide and is one of the leading causes of death in Europe. [1,2] A clinical definition of SCD is abrupt circulatory collapse due to CV causes that occurs within 1 h of onset of symptoms or within 24 h in case of unwitnessed death.[3] SCD is often attributed to ventricular arrhythmia (VA), i.e., ventricular tachycardia (VT) and ventricular fibrillation (VF). [2-5]

Pathophysiology of VA is complex as it is believed to be triggered by an interaction between an underlying substrate, particularly among patients that have increased CV risk profile and subclinical (undiagnosed) or diagnosed structural heart disease, and a trigger or a transient event that induces fatal ventricular arrhythmia (**Figure 1**). [1]

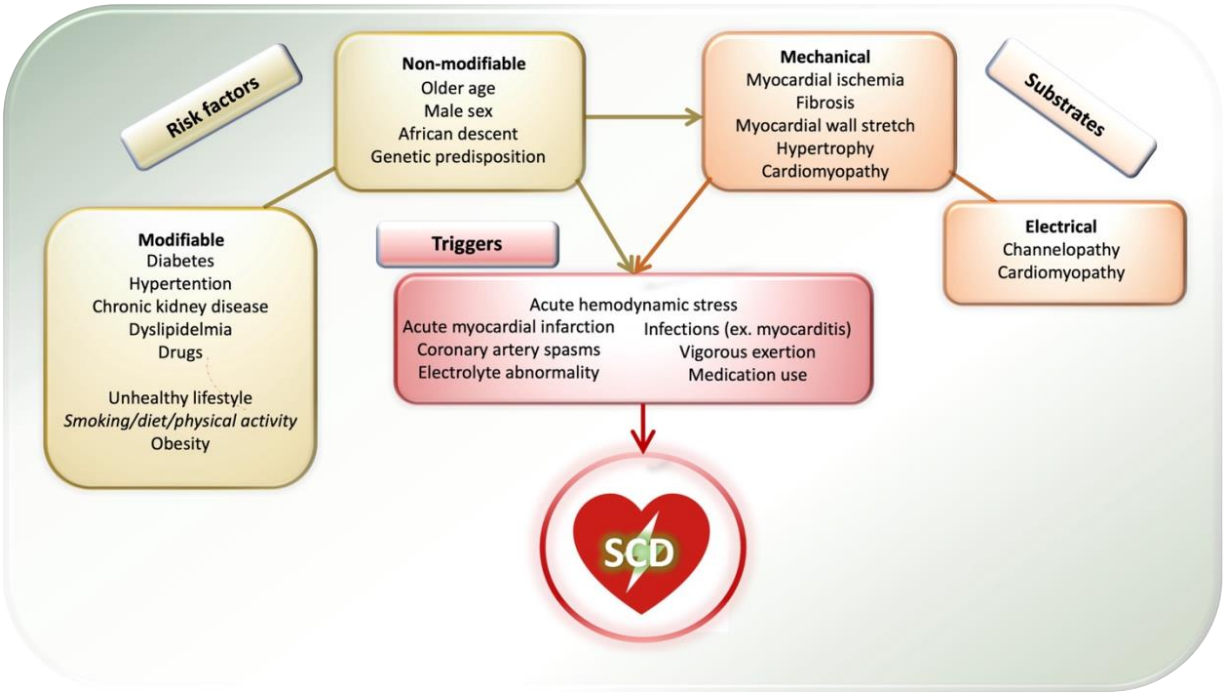

Figure 1 Risk factors, substrates and triggers of ventricular arrhythmia and sudden cardiac death. (Own figure)
SCD: sudden cardiac death.

A heterogeneous spectrum of diseases has been associated with an increased risk of SCD, and it is often the first manifestation of the disease, making the prediction of SCD in individual patients challenging. [4] The main cause of SCD is coronary artery disease (CAD), [4,6] and the proportion of CAD-related SCD increases with age.[4,6] Channelopathies,

cardiomyopathies, myocarditis, and coronary anomalies are the most common causes of SCD in the younger population (<50 years), while CAD dominates from the fourth decade of life.[7-11] Hence, structural heart diseases such as CAD, HF and valvular heart disease are common underlying causes for SCD in adult patients.[11] In all age groups, men have a higher incidence of SCD compared to women, also after adjusting for risk factors of CAD. [1]

The sudden and unexpected nature of SCD contributes greatly to the low survival rate of around 8% at hospital discharge in Europe, 3-6% in Asia, 11% in the USA and 12% in Australia and New Zealand for out-of-hospital cardiac arrest.[12] In-hospital cardiac arrest survival rates at 30 days or to hospital discharge have been reported to be 15-34% in Europe and around 25% survival to hospital discharge in the US. [12] Given the poor survival rates after cardiac arrest worldwide, a preventive approach is essential, which is greatly dependent on adequately predicting VA.

The best-known current predictors for future SCD are prior episodes of VA and the severity of HF as reflected in a reduced left ventricular ejection fraction (LVEF) and high New York Heart Association (NYHA) functional class. Hence, according to current European Society of Cardiology (ESC) guidelines patients with prior VA or severe HF should all be offered ICD if the estimated life-expectancy is >12 months. [3] In more detail; ICD treatment is recommended for primary prevention in patients with LVEF ≤35% and NYHA functional classification II-III after at least 3 months of optimal guideline-directed medical therapy. Patients with previously documented VF or sustained VT with hemodynamic consequence should be offered ICD as secondary prophylaxis, given that they have a life expectancy>12 months with good quality. [3] Solely following guidelines recommendations on ICD treatment however poorly discriminates people at risk [13,14], especially in the many cases where SCD is the first manifestation of cardiac disease, e.g., subclinical HF, CAD, cardiomyopathies, and channelopathies. While NYHA class and the degree of LVEF impairment are powerful predictors of the risk of mortality, it lacks specificity in predicting arrhythmical death. [15] Moreover, the pivotal trials for primary prevention of ICD in HF were undertaken more than two decades ago. Since then, the risk of SCD has declined due to improvements in therapy. [16] This underlines the unmet need for contemporary assessment of risk prediction to identify individuals at risk of developing SCD.

1.2 Ventricular arrhythmias

VT and VF (**Figure 2**) are the main causes of SCD. [4,6] It is frequently associated with CAD, but also occurs in a variety of other structural non-ischemic conditions and arrhythmogenic entities that can affect the heart muscle or its electrical function. [6]

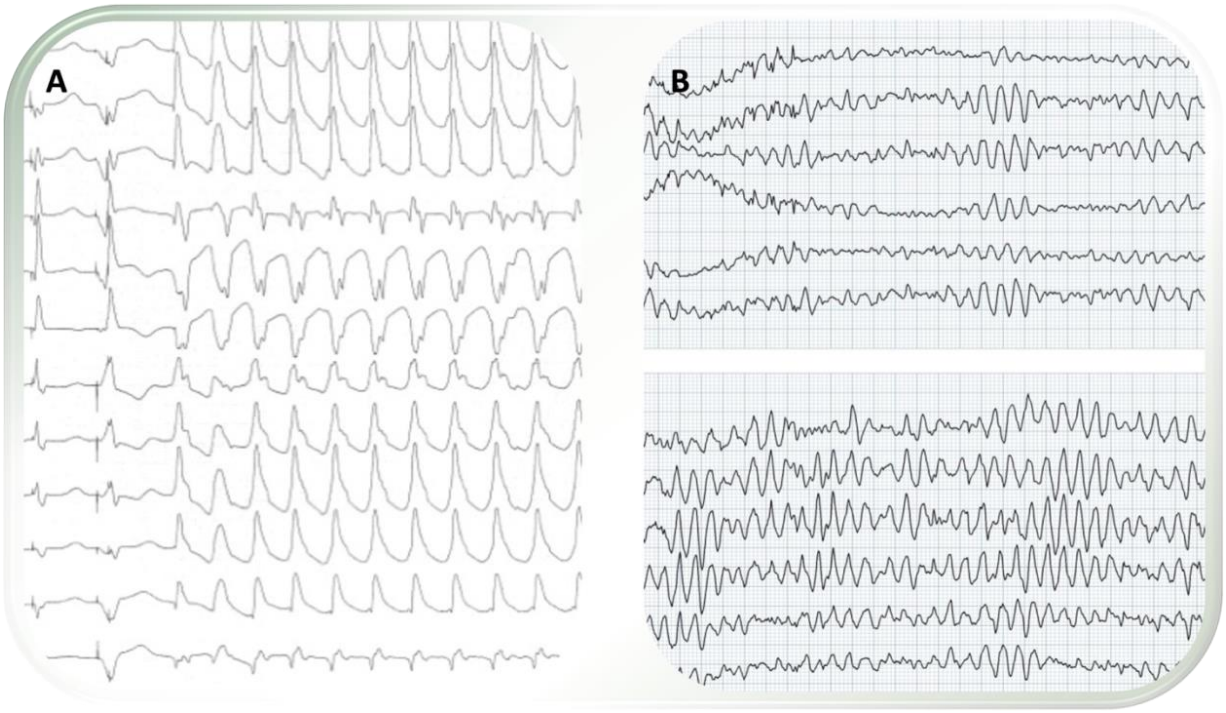

Figure 2

A. 12-lead ECG recording of ventricular arrhythmia. Figure is provided by electrophysiologist dr Harald Kjekshus at the Department of Cardiology at Akershus Univeristy Hospital.
B. 12 lead ECG recording of ventricular fibrillation. Figure is reproduced from ecgguru.com, published by Andreas Röschl. Permitted use without copyright for noncommercial use.

VT is an ectopic ventricular rhythm that lasts for at least 3 consecutive beats with a rate higher than 100 beats per minute. [17] VT can be classified according to duration as non-sustained, meaning it lasts <30s and terminates spontaneously without leading to hemodynamic instability, or sustained lasting for >30s or requiring intervention, which may or may not lead to hemodynamic instability. [3,17] The susceptibility for developing hemodynamic instability is dependent on general myocardial function, presence of CAD, and other comorbidities as well as genetic predisposition and frequency of the ventricular arrhythmia. [18] VT can further be classified according to morphology as monomorphic and polymorphic, based on whether the morphology of the QRS complexes is consistent during the arrhythmic episode. [3,17] The morphology of QRS complexes during VT is important to determine the location of the putative focus.

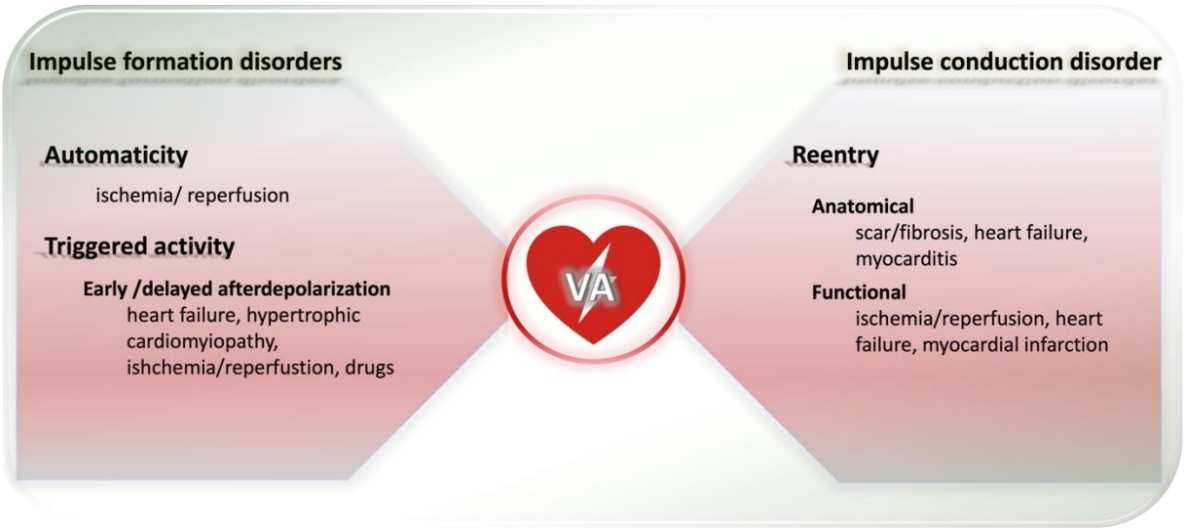

Figure 3. Electrophysiological mechanisms of cardiac arrhythmias, divided into impulse conduction and formation disorders, with possible triggers. (Own figure)
VA: ventricular arrhythmia.

From an *electrophysiological standpoint*, mechanisms that can trigger and maintain VA can be caused by a disorder of impulse generation or a disorder of impulse conduction, or both (**Figure 3**). [19-21] *Automaticity* is a disorder of impulse generation. The ability of cardiac tissue to spontaneously depolarize and initiate an action potential without the need for prior external electrical stimulation is physiologically present in the sinoatrial node and subsidiary pacemaker cells (atrioventricular node as well as the His–Purkinje system) but not in other cardiac cells. [19-21] Automaticity in other cardiac cells can trigger VA, especially in case of ischemia and reperfusion conditions, [21] and can trigger premature ventricular contractions that in turn can lead to re-entry VA. [19] *Triggered* activity is another disorder of impulse generation resulting from premature activation of cardiomyocytes by afterdepolarization, which is fluctuation in membrane potential dependent on the calcium current and the preceding action potential. [22,23] While automaticity is considered a self-generating arrhythmia arising without the need for any prior electrical stimulation, triggered activity arrhythmias are initiated in response to a preceding impulse, a trigger.[19,23] Such triggers can be disturbed calcium handling, as it can trigger VA directly by generating afterdepolarization or indirectly by modulating action potential time course and duration. [24] When the amplitude of early or delayed afterdepolarization brings the membrane to its threshold potential, it results in a triggered response of spontaneous action potential, [25] potentially leading to VA. Arrhythmias caused by triggered activity can be observed in hypertrophy and HF, triggered by early or delayed afterdepolarization. [25-27]

Re-entry is a disorder of impulse conduction in which an action potential fails to extinguish itself and persists to re-excite a region that has recovered from its refractoriness. [19,21] It is a self-sustaining arrhythmic disturbance in which an impulse

propagates in a self-perpetuating closed circus-like loop manner. [19,20] Reentry can be anatomical, caused by a structural disturbance such as in the case of scar, or functional, caused by heterogenous disturbances in the electrophysiological properties of the cardiac tissue such as in surviving myocardial tissue intercepted between myocardial scar post-MI, or a combination of both forms, such as in the case of AMI. [19,21,28] In general, re-entrant tachyarrhythmias are the most common pathophysiological type of arrhythmia secondary to ischemia. [19,21]

From a *disease perspective*, CAD is the leading cause of VA leading to SCD, especially for out-of-hospital SCD cases. [29-31] In acute CAD, leakage of potassium can lead to depolarizing of myocytes in the ischemic region providing a substrate for reentry, [6,32,33] while injury currents depolarizing adjacent nonischemic cardiac tissue, can trigger VA through increased- or abnormal automaticity. [19,31] For these patients, pharmacological treatment and more importantly revascularization therapy have been shown to improve survival. [34,35] In chronic CAD, which in many cases is undiagnosed, changes in the structural or functional cardiac or vascular system may lead to a transition from a stable CAD to an unstable pathophysiological state triggering VA. [36] These changes can be caused by transient ischemia that lead to myocardial perfusion variations acting as a substrate for VA by re-entry and triggered activity. [36] This can occur in patients with high-grade chronic lesions and may have a subsequent inability to meet adequate flow requirements under specific conditions with acute changes in the flow supply-demand such as the case during exercise (increased demand) or tachycardia (reduced supply), while unstable plaques are susceptible to transient spasms in the coronary artery triggering an arrhythmia. [36] Generally, for patients with ischemic CAD and episodes of VA or cardiac arrest revascularization in addition to ICD treatment has been suggested to improve survival. [37-39] Revascularization of patients with scar-mediated VA, where the scar is believed to constitute the root of reentry circuits, has not been shown to be associated with lower incident of VA. [40,41] In contrast, such foci for ventricular arrhythmia should be considered for treatment with ablation, given that the focus is available for ablation catheters. [42]

Anatomical myocardial alternations, such as in the case of myocardial fibrosis due to scar formation or myocardial stretch, can provide an anatomical substrate for reentry and can lead to spontaneous depolarization and triggered activity triggering VA. [4344-46] In patients with HF, many factors can predispose to the development of VA such as structural (ex. scar and stretch of myocardium), metabolic (ex. neurohormonal activation), and electrophysiological changes (ex. changes in action potential and calcium handling). These different anatomical, metabolic, and electrophysiological changes can lead to a number of arrhythmic mechanisms. Accordingly, reentry, automaticity, and triggered activity are all potential underlying mechanisms for VA in patients with HF. ESC guidelines emphasize ablation strategies for the treatment of patients with VA and suspected scar as the underlying etiology. [3]

1.3 Implantable cardioverter defibrillator

ICD is useful in preventing SCD and is an integral part of treating patients with high primary or secondary risk of VA and SCD. Several trials have demonstrated ICD treatment to be superior to medical therapy in patients who have experienced VA (secondary prevention). [47-49] Meta-analyses of 3 ICD trials in secondary prevention demonstrated a 28% reduction in mortality (HR 0.72: 95% CI 0.6–0.87, P <0.001) in the group with medical therapy and ICD compared to the group receiving medical therapy alone, which was almost entirely due to reduction of arrhythmic death in the ICD group. [50] Accordingly, current guidelines recommend ICD treatment for the secondary prevention of SCD, in patients with documented VF or hemodynamically not-tolerated VT in the absence of reversible causes. [3]

Similarly, trials have demonstrated that ICD treatment has an established role for the primary prevention of SCD in patients with HF with reduced LVEF (HFrEF). [51,52] In the MADIT trial, patients treated with ICD had a 31% lower mortality rate (14.2%) compared with the conventional-therapy group (19.8%). [51] Similarly, in the SCD-HeFT trial, ICD therapy reduced mortality by 23% while amiodarone therapy had no favorable effect on survival in patients with symptomatic HF with LVEF ≤35%. [52] Based on these studies, current ESC guidelines recommend ICD treatment for the primary prevention of SCD in patients with symptomatic HF and a LVEF ≤ 35%. [3] However, while the survival benefits of ICD treatment in HFrEF patients with ischemic etiology of heart disease is well established, there has until recently been limited evidence for the benefit of ICD treatment in patients with non-ischemic etiology. Improvements in HF therapy have reduced the incident rates of SCD, and it remains unknown whether the effect of ICD persists in contemporary care. [53-55] To close this knowledge gap, the DANISH trial was designed and conducted at all centers (5 sites) with ICD implantation in Denmark between 2008 and 2014. The trial randomized 1116 patients with non-ischemic HF, LVEF ≤ 35%, and NYHA class II-III, to ICD or no ICD treatment in addition to optimal medical therapy for HF. [56] After a follow up of 5.6 years, there were no survival benefits in patients treated with ICD (HR 0.87 [95% CI 0.68-1.12] p=0.28), although there was a reduction in the rate of SCD (HR 0.50 [95% CI 0.31-0.82], p=0.005). [56] Taking these findings into consideration, and given the limited evidence supporting ICD treatment in patients with non-ischemic HF, ESC guidelines now have a lower class recommendation (class IIa), for ICD for primary prevention of SCD in patients with non-ischemic HF. [3]

2. Introduction to markers studied in the PhD project

2.1 QRS fragmentation

ECG is a recording of the cardiac electrical activity where each ECG signal reflects a specific cardiac cycle. ECG is an important tool in the diagnosis of different types of CVD and is fundamental for diagnosing VA. [3,57] The QRS complex in the ECG reflects ventricular depolarization, which is a sensitive phase where abnormalities in the electrical conduction can be a potential arrhythmical trigger. [57,58] Fragmentation of the QRS complex (fQRS) (**Figure 4**), which is believed to be triggered by myocardial fibrosis, is recognized as a marker of altered ventricular depolarization, and has been suggested to be associated with the risk of VA and SCD. [59]

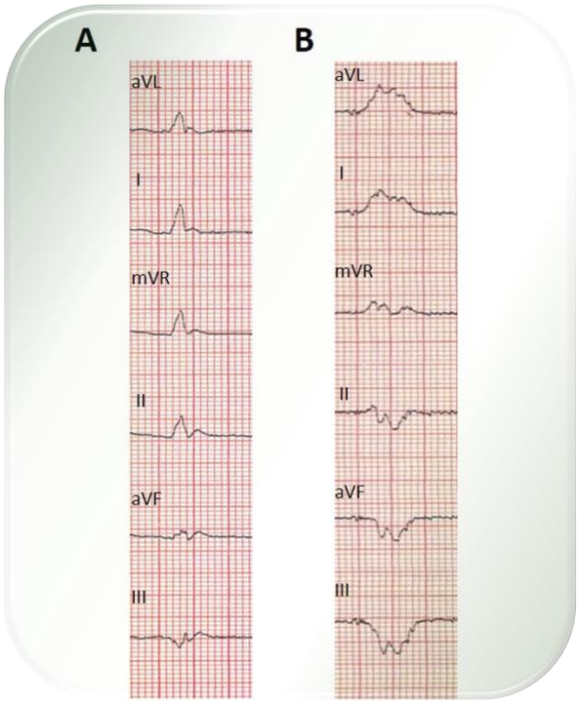

Figure 4. Example of fragmented QRS in a patient with narrow QRS (panel A) and wide QRS (panel B), with paper speed 50 mm/s. (Own figure)
A. Patient with QRS fragmentation in narrow QRS in both lateral- and inferior wall (i.e., additional R-wave).
B. Patient with QRS fragmentation in wide QRS in lateral wall (i.e., >2 notches in R waves), and also in inferior wall to some extent (i.e., >2 notches in S waves)

fQRS is defined as the presence of additional notches or fragmentation in the QRS complex, for which the criteria differ depending on QRS duration and ECG rhythm (native or PM-rhythm) (**Figure 5**). [60] For native QRS rhythms, fQRS was defined by Das et. al. as the presence of >1 R' (fragmentation), an additional R-wave (R') or the presence of or notching in the downslope of the S-wave. Due to the risk of over-interpretation of fQRS in ECG with incomplete bundle branch block (BBB), these have consistently been excluded in prior studies. [61-63] For native wide QRS complex (>120ms) and for ECG rhythm with BBB, fQRS was defined as RSR' patterns with >2 notches in the R-wave or S-wave, or >2 R-waves. [60,63] In paced PM rhythm, fQRS is defined as is the presence of >2 R' or >2 notches in the S-waves. Common for the aforementioned criteria is that fQRS must be present in two contiguous leads corresponding to a major coronary artery territory to be of significance: [62,63] I, aVL, V6, represent the lateral leads, V1-V5 the anterior leads and II, III, aVF the inferior leads. [62,63] In Paper I we used these criteria to define fQRS.

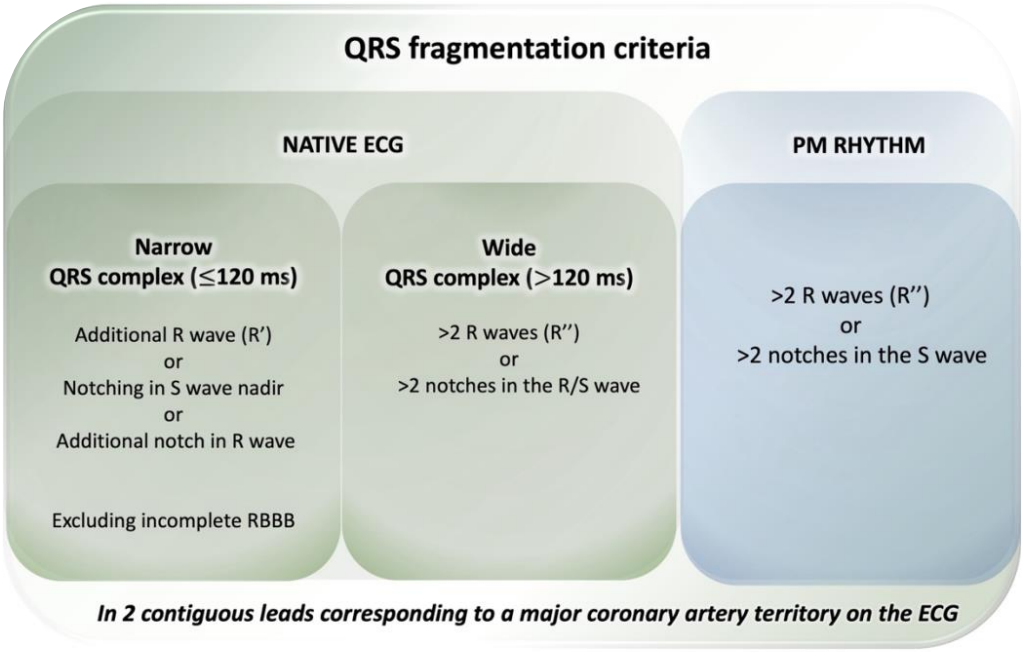

Figure 5 Criteria for defining QRS fragmentation depending on QRS duration and whether it is a native or PM-rhythm. (Own figure)
RBBB: right bundle branch block

2.2 Natriuretic peptides

The natriuretic peptide (NP) family includes A-type (or atrial) Natriuretic Peptide (ANP), B-type Natriuretic Peptide (BNP), and C-type Natriuretic Peptide (CNP). [64,65] BNP is synthesized as pre-proBNP from which proBNP is derived after enzymatic removal of the signal sequence. During or after release from cardiomyocytes, proBNP is believed to be enzymatically cleaved into its biologically active C-terminal fragment, BNP, and the inactive N-terminal fragment, NT-proBNP (**Figure 6**).[66,67] However, intact, uncleaved proBNP is also found circulating in significant amounts. proBNP is synthesized and released by cardiomyocytes in response to pressure or volume overload, myocardial wall stress, hypoxia, and neurohormonal activation.[66,67] In physiological conditions, NT-proBNP and BNP are produced in low concentration from both atrial and ventricular tissue.During cardiac pathological states, such as increased ventricular wall stress, the ventricles become the dominant chamber for proBNP production. [68,69] Both BNP and NT-proBNP have been shown to be useful for diagnosing HF [70] [71] and to risk stratify patients across a number of different conditions. [72-74] NT-proBNP, is more stable with less biological variability and has longer half-life and slower rate of degradation compared to BNP.[75] Cut-points and absolute values of NT-proBNP and BNP should not be used interchangeably.[75]

The NPs have a number of counteracting, positive effects on cardiac structure and cardiovascular homeostasis. As an example, structural or functional abnormalities of the failing heart lead to increased intracardiac pressures and/or inadequate cardiac output, [76] triggering the upregulation of the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone system (RAAS).[77] Initially, the upregulation of SNS and RAAS acts as a compensatory mechanism that helps to maintain homeostasis, however, in chronic HF this prolonged upregulation results in cardiac remodeling and eventually HF. [78] To counteract the effects of prolonged activation of RAAS and SNS, NPs are secreted in attempts to restore physiological circulatory conditions and to limit the cardiac remodeling process through its natriuretic and diuretic action, as well as inhibiting the RAAS activation (**Figure 6**). [77,78]

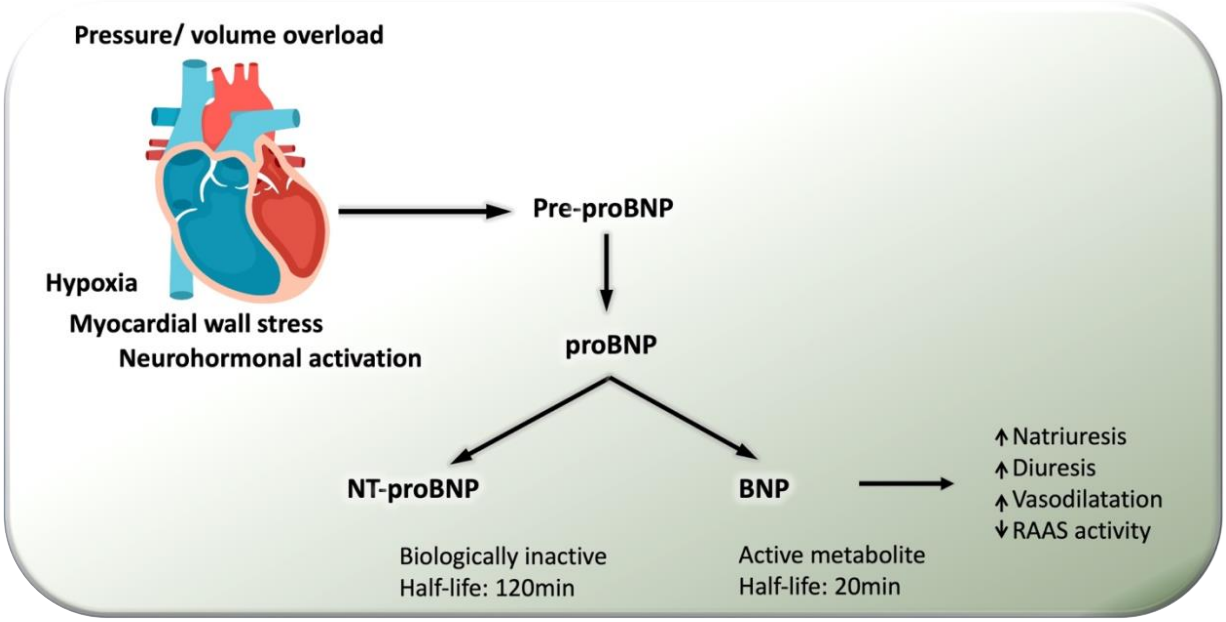

Figure 6 Schematic illustration of the production/secretion pathways of natriuretic peptides in heart failure with their main actions to reduce water and salt retention and vasoconstriction. (Own figure)
NT-proBNP: N-terminal pro-B-type natriuretic peptide; BNP: B-type natriuretic peptide
RAAS: renin-angiotensin-aldosterone system

Measurement of NT-proBNP or BNP is recommended for diagnosing HF (class 1B recommendation in the European HF guidelines, and class 1A recommendation in the American HF guidelines) and is particularly useful because of the high negative predictive value. [76,79] These biomarkers have also been shown to be highly useful in assessing HF disease progression and prognosis. [76,79] Measurements of NPs have been incorporated into the clinical definition of HF but should always be used together with clinical history and examination and later also cardiac imaging. According to the clinical definition used in the Universal Definition of HF, HF is a clinical syndrome with (current or prior) symptoms and/or signs secondary to structural or functional cardiac

abnormalities with elevated NPs and/or objective evidence of cardiogenic pulmonary or systemic congestion at rest or with provocation. [80] European and American guidelines define HF as a syndrome with symptoms and signs of HF, evidence of cardiac abnormalities with LV diastolic dysfunction, raised LV filling pressures and/or increased NPs. [76,79] These guidelines further classify HF by LVEF into HF with reduced EF (HFrEF) with LVEF ≤ 40%, HF with mildly reduced EF (HFmrEF) with LVEF 40-50% and HF with preserved EF >50% (HFpEF).[76,79] NPs have lower concentrations in patients with HFpEF compared to patients with HFrEF. [81] In HFpEF, around 20% of patients have NPs within the reference limits, but it still serves as a great diagnostic tool and is part of the recommended diagnostic assessments of patients with HF regardless of LVEF. [76,79,82]

Beside their extensive documentation and the clinically established diagnostic role, NPs are important for monitoring patients with chronic HF as higher NPs concentrations have been found to be associated with disease severity and prognosis. [77] Concentrations of NPs are associated with the risk of cardiovascular events, in particular heart failure events and CV mortality in low-risk cohorts. [7483,84] NPs have also been suggested to associate with SCD risk [85-87 88] However, these studies have been limited by low number of patients included, [89] short follow-up period, [87,90] or including only a specific subgroup of patients with ICD. [90-92 88]

2.3 Cardiac troponin

Troponin is a protein complex that is part of the contractile apparatus of cardiomyocytes and myocytes. Troponin molecules are connected to the thin filament in the cytosol of the cardiomyocyte together with a double helix of actin monomers (basis) and connected tropomyosin molecule (**Figure 7**). [93-95] The troponin complex contains three subunits, troponin C (TnC), which is common to all muscle types, and the two cardiac specific troponins; cardiac troponin I (cTnI) and cardiac troponin T (cTnT). [93,96] While cTnI is the inhibitory component of the troponin complex, and TnC is the Ca^{2+} binding component, both play a role in regulating muscle contraction. cTnT has a more structural function by function as an anchor for the troponin complex to the thin filament. [93,94]

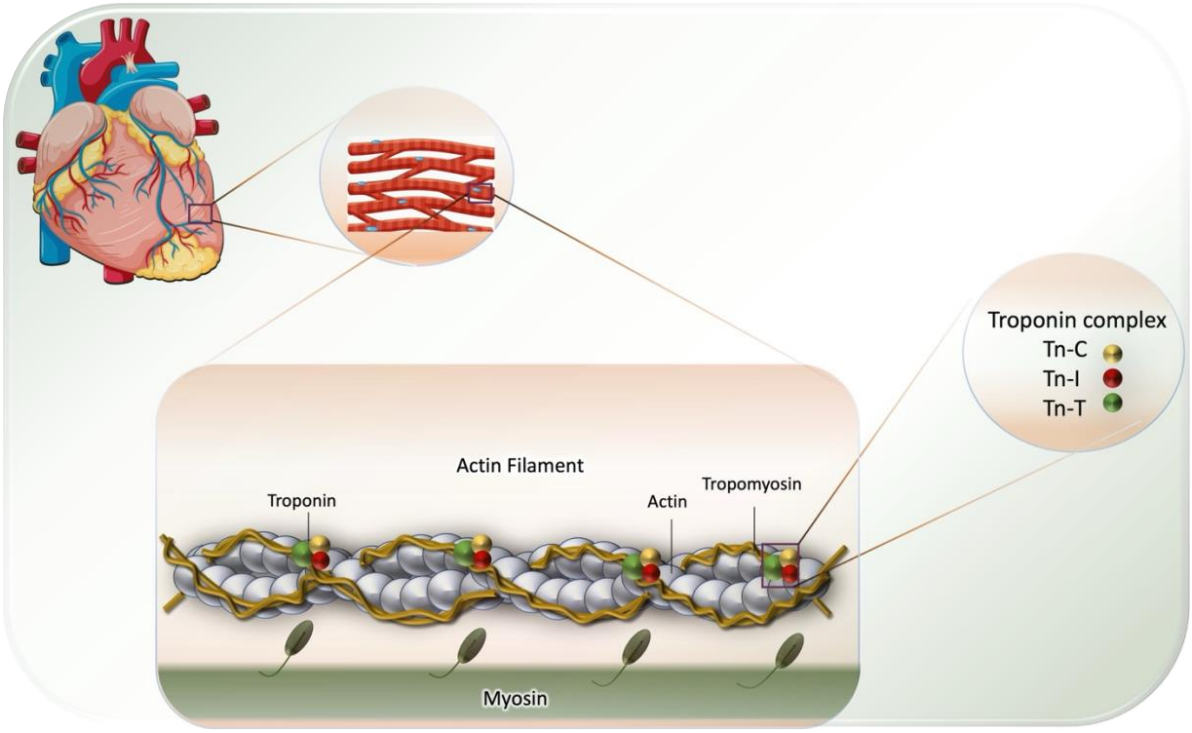

Figure 7 Illustration of the contractile apparatus of cardiac myocytes. Illustrating thin (actin), and thick (myosin) filaments, with the troponin complex embedded in the thin filament. (Own figure) Tn-C: troponin C; Tn-I: troponin I; Tn-T: troponin T

Troponin molecules are mainly released into the circulation due to disease-induced modifications/degradations and it circulates both as intact and degraded troponin products.[97] Degradation of the thin filament and its proteins, including troponin, has generally in the clinical setting been considered a marker of myocardial injury.[93] Myocardial injury leads to degradation of troponin molecules and increases the permeability of the cardiomyocyte cell membrane, which promotes the release of troponin from cardiomyocytes. [97] Increased permeability of the cardiomyocyte cell membrane may result from damage to the membrane due to ischemia or mechanical stress on the myocardium. [97] Alternations in the composition of proteins in the contractile apparatus, including troponins, due to pathological conditions that influence the overall contractile function of the heart.

Methods to measure circulating troponin have markedly improved over the last decades. Cardiac specific troponins (cTn) can now be detected by using highly specific monoclonal antibodies that bind to epitopes of cTnI and cTnT. [98] Highly sensitive cardiac troponin (hs-cTn) assays are required to have a coefficient of variation (CV) <10% at the 99th percentile, and to be detectable in >50% of the healthy population, in both men and women. [99] Concentrations above the 99th percentile upper reference limit (URL) in the general population is considered pathological and indicative of myocardial injury. [99] hs-

cTn is widely utilized today and is an obligate criterion to diagnose MI according to the 4th WHO classification for MI. [100] High cardiac troponin concentrations are also accurate for predicting poor outcomes, both in the setting of acute elevations secondary to MI [100-102], in other CV conditions [103,104], and also in non-cardiac diseases such as in sepsis [105], COPD [106], and acute pulmonary embolism [107] as well as in the general population. [108]

Chronically elevated hs-cTn can be indicative of chronic myocardial injury secondary to cardiac and non-cardiac causes of chronic myocardial injury. [109] Cardiac causes of elevated cTn can be chronic HF, hypertensive heart disease, cardiomyopathies, and CAD. [109] cTn is frequently elevated in patients with chronic HF, often in the absence of ischemia. [110] Although the exact mechanisms are unknown it seems closely connected to LV mass and possibly reflective of ongoing myocardial injury, independent of ischemia. [111] For example, myocardial wall stretch can lead to leakage of cTn due to cell membrane integrity loss. [111]

Chronic cTn elevations reflecting a steady disease state with myocardial injury, have been associated with a higher rate of adverse cardiovascular outcomes and poor prognosis in different patient populations, including non-ischemic conditions. [111-115] Seliger et al. found cTn to be associated with incident HF, CAD and cardiovascular death, and with a non-ischemic myocardial fibrosis pattern on late gadolinium enhancement in the general population.[116] These findings demonstrate that ischemia is not necessarily the etiology of myocardial injury reflected by higher cTn levels. In patients with stabile chronic HF, higher levels of cTn have been found to be associated with adverse cardiovascular risk such as HF worsening, HF hospitalization, all-cause mortality and cardiovascular mortality. [111,113] Among patients with pulmonary arterial hypertension, cTn has been shown to predict mortality, worsen functional class, and was found to be related to systolic right ventricular dysfunction. [114] cTn levels are associated with major cardiovascular events, cardiovascular mortality and all-cause mortality among patients with chronic kidney disease. [115]

2.4 Biomarkers of inflammation

Inflammation is a complex, and highly regulated process that is essential to host defense and tissue repair but can be maladaptive when chronically activated such as in the case of coronary atherosclerosis and HF. [117] Chronic inflammation has a key role in all phases of atherosclerosis and can further be influenced by several risk factors such as smoking, dyslipidemia, DM, and genetic predisposition. [117] In HF, chronic inflammation can trigger a vicious circle of cascade of events promoting further cardiac remodeling, which in turn promotes and sustains the inflammatory response. [118,119]

Systemic inflammation can be detected by measuring different biomarkers in the blood. growth differentiation factor 15 (GDF-15), interleukin 6 (IL-6), and C-reactive protein (CRP) are proteins that are considered markers of inflammation, although they also reflect several other pathophysiological entities. These biomarkers have been recognized to have special importance in cardiac disease as they consistently have been

found associated with development and disease progression in patients with CAD and HF. [120-123]

GDF-15, also called macrophage inhibitory cytokine 1, is an inflammatory cytokine belonging to the transforming growth factor-β (TGF-β) superfamily.[124] TGF-β superfamily is composed of proteins that are essential regulators of diverse cellular functions.[124] GDF-15, and other proteins of the TGF-β superfamily, are produced as inactive precursor proteins, which are cleaved into their active form in response to stimulation, such as inflammation, hypoxia, and oxidative stress. [124,125] In physiological conditions, GDF-15 is expressed in most tissues, including cardiomyocytes, at low concentrations. Expression of GDF-15 is prominently upregulated following several pathological conditions, such as inflammation, tissue injury, and remodeling. [124,126] Previous studies have demonstrated that GDF-15 is elevated in patients with stable CAD and HF and reflects the severity of the disease. [127-129] GDF-15 is strongly associated with the risk of cardiovascular events and mortality in most clinical settings. [129-132]

IL-6 is a cytokine produced in response to inflammation, tissue injury, and oxidative stress [133,134]. IL-6 has an important role in mediating inflammation, and stimulating acute-phase inflammatory response. [135] IL-6 is also the primary stimulant for inducing hepatic synthesis of CRP. [136] CRP and IL-6 both participates in the systemic response to inflammation, however in the acute setting, IL-6 peaks faster than CRP and has a shorter half-life. [137,138] This seems logical, since IL-6 is responsible for stimulating the release of CRP, [136] and with a slow synthesis and release of CRP, IL-6 is to be detected significantly earlier than CRP in acute settings. [139] Higher levels of IL-6 and CRP have been demonstrated to be independently associated with HF disease severity and prognosis, [140-143] with atherosclerotic processes, [144,145] and to help predict adverse cardiovascular events. [121,122,146-148] IL-6 and CRP have also been found associated with all-cause mortality in patients with CAD[121,122] and HF. [142,143] Although IL-6 is the main inductor of CRP synthesis, the correlation between circulating IL-6 and CRP concentrations is only poor-to-moderate, [149] and IL-6 has consistently been demonstrated to be a more sensitive biomarker, with stronger associations with CV events, mortality and generally poor prognosis than CRP in different clinical settings. [150-154] IL-6 and CRP also seems to be promising therapeutic targets in HF, and there is a large ongoing phase 3 trial testing the effect of IL-6 inhibition in patients with HFpEF (ref HERMES trial, clinicaltrials.gov)

3. Aims of the thesis

The overarching aim of this thesis was to identify markers that can improve the risk prediction for VA and SCD. The specific research aims where as follows:

- Paper I: to assess whether fQRS is associated with incident VA in patients treated with ICD.
- Paper II: to assess the association between concentrations of NT-proBNP and incident VA in patients treated with ICD.

- Paper III: to assess the association between concentrations of cTnT, GDF-15, IL-6, and CRP in association with incident VA in patients treated with ICD.

The secondary aim was to assess the association between fQRS, NT-proBNP, cTnT, GDF-15, IL-6, and CRP and the risk of HF hospitalization and all-cause death.

4. Materials and methods

4.1 Study design/population

The articles in the thesis are based on data from the SMASH 1 Study (Scandinavian Multicenter study to Advance risk Stratification in Heart disease - ventricular arrhythmia), which was designed to identify predictors of VA from circulating biomarkers and resting ECG recordings. The study was conducted in an observational, prospective, multicenter fashion and included 495 ICD patients recruited during their regular outpatient ICD controls at the Cardiology Departments of AUH and SUH, from August 2016 to March 2018. A follow-up visit was conducted after 1-2 years from the baseline visit. The study was approved by the Norwegian Regional Ethics Committee (#2015/2080). The SMASH 1 Study was registered at clincialtrials.gov (NCT02864771) prior to study start.

Patients were eligible for inclusion if they were currently treated with an ICD, were ≥18 years old, and were able to provide informed, written consent. We excluded patients with conditions that could impair their ability to participate in the study (i.e., severe medical condition and/or short life expectancy), history of drug- or alcohol abuse the last 12 months before inclusion, history of non-compliance to medical management, and participation in other interventional trials.

4.2 Clinical assessment and medical history

Relevant medical history such as HF, DM, CAD, and cardiomyopathy, as well as symptoms including NYHA functional class, and medication use were obtained through a structured and standardized interview as well as by conducting a thorough review of electronic medical records. Information regarding the most recent LVEF measured by echocardiography or cardiac magnetic imaging was recorded. Glomerular filtration rate (eGFR) was estimated based on creatinine concentrations obtained from routine blood sample measurements registered in the medical records.

At both the baseline visit and at the follow-up visit, patients underwent a standardized physical examination, which included heart rate and blood pressure measurements after 5 minutes of rest where we used the average of measurements #2 and #3. Body mass index (BMI) was calculated by dividing body weight (kg) over height squared (m).

4.3 ECG analyses

A resting 10 second 12-lead ECG was obtained by experienced study nurses using ECG machines that was in routine clinical use at the study sites (Mortara 350/380 with filter 150 Hz, AC filter 50Hz, 50mm/s, 10mm/mV and Schiller AT-110 with filter 150 Hz, AC

Filter 50Hz, 50mm/s, 10mm/mv). All ECG recordings were analyzed and adjudicated by two independent physicians blinded to outcome data, and disagreement on the adjudication was resolved by consensus, involving a third adjudicator if needed.

Several ECG parameters were obtained either by manual or automated measurements from patients' baseline ECG recordings. QRS duration and axis, corrected QTc time and T axis were obtained from automated measurements on ECG recordings. Q-wave amplitude was measured from the deepest pathological Q wave, at least present in 2 contiguous leads corresponding to a major coronary artery territory. QRS fragmentation was assessed as described earlier in the introduction of this thesis. T-peak to T-end is the distance between the maximum deflection of the T-wave (T-peak) and the returning point of the T-wave to the isoelectric line, which was measured using the "tangent" method [155], using the maximum value from all leads (**Figure 8**).

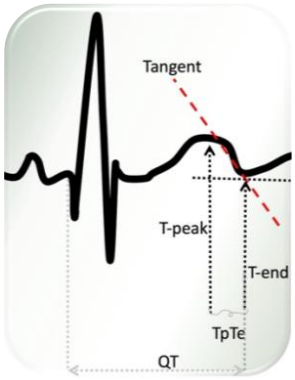

Figure 8 Measurement of T-peak to T-end using the "tangent" method. (Own figure)
TpTe: T-peak to T-end interval

4.4 Biochemical analyses

Blood samples were obtained by venipuncture at the baseline visit and at the follow-up visit by experienced study nurses. The blood samples were temporarily stored at 4°C and centrifuged at 2000 G for 10 minutes before they were transferred into aliquots that were stored at -80°C. All blood samples were transported for long-term storing in the study biobank at AUH. Serum blood samples that had not previously been thawed were later used to analyze the studied biomarkers: NT-proBNP, cTnT, GDF-15, IL-6, and CRP by the electrochemiluminescence immunoassay Elecsys on the Cobas e 801 platform (Roche Diagnostics, Rotkreuz, Switzerland) at the core laboratory at AUH.

The coefficients of variation (CV) as reported by the manufacturer, and the analytical range (lowest to highest level of detection) for NT-proBNP, cTnT, GDF-15, IL-6 and CRP are presented in the table below (Table 1).

Biomarker	Coefficients of variation (CV)	Analytical range
NT-proBNP	2.5% at 127 ng/L and 1.3% at 1706 ng/L	5-35 000 ng/L
cTnT	3.9% at 11.8 ng/L and 3.0% at 89 ng/L	3-10000 ng/L
GDF-15	1.3% at 472 pg/mL and 1.1% at 19368 pg/mL	400-20000 pg/mL
IL-6	4.9% at 6.4 pg/mL and 1.4% at 189 pg/mL	1.5-5000 pg/mL
CRP	3.3% at <5mg/L and 1.5% at ≥ 5 mg/L	0.3-350 mg/L

4.5 Outcome measure

The outcomes in the SMASH-1 study was prespecified and registered at clinicaltrials.gov prior to the study start (ref SMASH 1 trial, clinicatrials.gov). The primary outcome was incident VA, defined as sustained VT or VF (>100 beats per minute >30 sec), or VA resulting in appropriately delivered ICD therapies (electrical shock or antitachycardia pacing [ATP]). These arrhythmic events were obtained by review of ICD-interrogations to include ICD-recorded events as well as electronic hospital records that included clinically recorded events (e.g., VA episodes that fall outside programmed monitor/treatment zones) as diagnosed by experienced cardiac electrophysiologists. Validation of these records was performed by a thorough review of all ICD recordings and electronic health care records to ensure that real arrhythmic events and appropriate ICD therapies were separated from artifacts and inappropriate ICD therapies. We only included validated, real arrhythmic events and appropriate ICD therapy as outcomes in the SMASH 1 Study.

The secondary outcomes were death from any cause and hospitalization for HF. These events were obtained by a thorough review of the electronic healthcare records, with linkage to data from the Norwegian National Death registry. HF hospitalization was defined as hospital admissions where HF was the main reason for hospitalization, and this was adjudicated by an experienced physician from the study team.

4.6 Statistical analyses

Statistical software

Statistical analyses performed in the first paper was performed using Stata software version 16 (Statacorp., College Station, Texas, USA), and for papers II and III we used the updated version 17.

Standard statistical tests

Variables were assessed for normal distribution using a visual inspection of a histogram illustration of the data and the Shapiro-Wilk normality test. Baseline characteristics are reported as N (%) and median (Q1, Q3) for skewed and mean ± SD for variables demonstrating a normal distribution. Categorical variables were compared using the χ^2 test for binary variables, and for continuous variables t-test or ANOVA was used for the comparison of normally distributed/parametric continuous variables, and the Kruskal-Wallis-test for non-normally distributed/non-parametric continuous variables.

Regression analyses

The circulating biomarkers (NT-proBNP, cTnT, GDF-15, IL-6 and CRP) all had a non-normal distribution according to the Shapiro-Wilk normality test. Due to right-skewed distribution of biomarker concentrations, we transformed all biomarkers by the natural logarithm and used the log-transformed values in all regression analyses.

In papers II and III, we used linear and logistic regression to compare baseline characteristics of trends across quartiles of biomarkers. Linear regressions were used for continuous, normally distributed variables while logistic regression was used for categorical variables. Multivariable logistic regression analysis was used to assess independent predictors of fQRS in paper I, and multivariable linear regression analysis was used to assess independent predictors of higher baseline biomarker concentrations in papers II and III.

Cox proportional hazard regression analyses were used for time-to-first event for the exposure variables of interest. We used Cox regression models to examine the association between the presence of fQRS on baseline ECG and time-to-first event of incident VA and all-cause death in separate analyses in paper I, and to study the associations between baseline concentrations of biomarkers and time-to-first event for incident VA, HF hospitalization and death in separate analyses in papers II and III. The Cox regression analyses were performed in unadjusted (univariable) and adjusted (multivariable) models. The covariates used in the adjusted models were selected *a priori* based on established risk factors for VA: age, sex, BMI, CAD, HF, eGFR, and LVEF. We also adjusted for QRS duration, QRS axis, presence of Q-wave, and BBB in Paper I for patients with native QRS on baseline ECG.

Interaction analyses were performed to assess whether the association between fQRS/biomarker concentrations and incident VA was different according to ICD-indication (primary versus secondary prevention). In paper III interaction analyses were also performed to assess whether the association between cTnT and VA was different according to ischemic versus non-ischemic etiology.

Sensitivity, specificity, and assessment of discrimination
In paper I, we calculated sensitivity, specificity, and likelihood ratios for the ability of fQRS to predict VA. For the biomarker studies in papers II and III, Harrell's C-statistics was calculated to assess the performance of the studied biomarkers to discriminate between patients with and without events, using time-to-event. In paper III, we also calculated the additive prognostic value of GDF-15, IL-6, and CRP on top of cTnT. We performed this analysis by estimating the effect size of a basic clinical risk model plus cTnT alone against the effect size of a basic clinical risk model with the addition of the inflammatory biomarkers entered separately. We compared these different risk models using the likelihood ratio test.

Kaplan-Meier survival analysis

We developed Kaplan-Meier survival curves to graphically illustrate the event rate over time in patient groups classified by the presence of fQRS or not, and by quartiles of different biomarker concentrations. Kaplan-Meier survival curves were generated for all 3 papers in this thesis to visualize the proportion of patients with primary and secondary events over time. The log-rank test was used to compare the differences in survival between the different groups of the Kaplan-Meier curve.

Repeated measurements
Wilcoxon Signed Rank test was used to analyze changes in biomarker concentrations from the baseline visit to the follow-up visit in papers II and III. The relative change in concentrations of biomarkers was calculated by dividing the follow-up concentration with the baseline concentration. These calculated ratios of change were then log-transformed and analyzed in Cox regression models for incident events that occurred after the date of the follow-up visit (landmark analysis).

P-values of less than 0.05 was considered significant for all statistical tests. No adjustment for multiple hypothesis testing was performed.

5. Summary of the papers results

5.1 Paper I

We tested the hypothesis of an association between fQRS and risk of developing incident VA in **Paper I.** We analyzed 459 patients (after excluding 36 patients with missing baseline ECG, low-quality ECG recording, and patients with incomplete RBBB) treated with ICD and found that 52 (11%) of patients had fQRS. The presence of fQRS was strongly associated with the risk of developing VA during follow-up: HR 3.41 (95% CI 2.27-5.13), p<0.001 (**Figure 9**). This association was also statistically significant after adjusting for age, sex, BMI, CAD, HF, eGFR, ICD indication, and the following ECG variables in patients with native QRS: QRS duration, QRS axis, presence of Q-wave, and BBB. There was a trend for a stronger association between fQRS and VA among patients with a primary prevention ICD indication compared to patients with a secondary prevention ICD indication: HR 6.05 (3.16-11.60) versus HR 2.39 (1.41-4.04), respectively, p-for-interaction=0.047.

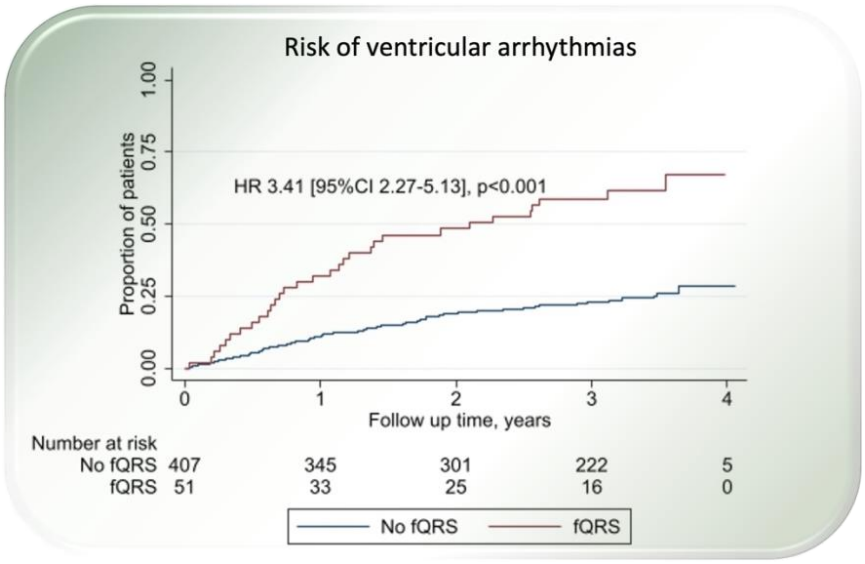

Figure 9 Survival analysis of time to ventricular arrhythmia in patients with and without QRS fragmentation (fQRS) present in the baseline ECG. (Own figure)

5.2 Paper II

We analyzed the association between NT-proBNP concentrations and incident VA in 490 patients treated with ICD in **Paper II**. In this study we found that NT-proBNP concentrations were elevated in the overall population (median 567 [Q1-Q3 203-1480] ng/L) and that higher concentrations were associated with increased risk of VA: HR 1.39 (95% CI 1.22-1.58) per log-unit increase, p<0.001, C-statistics 0.62). The association between NT-proBNP concentrations and incident VA persisted after adjusting for age, sex, BMI, CAD, HF, eGFR and LVEF in multivariable Cox regression analysis. There was a tendency for stronger association between NT-proBNP concentrations and incident VA in patients with secondary prevention ICD indication compared to patients with primary prevention ICD indication (p=0.06 for interaction). In a subgroup analysis among patients with secondary prevention ICD indication only, the association between NT-proBNP and incident VA persisted in adjusted models, the C-statistic was 0.71, and the risk was 7-fold higher for patients with NT-proBNP concentrations in the 4th quartile compared to patients with NT-proBNP concentrations in the 1st quartile (**Figure 10**). In contrast, in patients with primary prevention ICD indication, the association between NT-proBNP concentrations and incident VA was not significant in adjusted models and the C-statistic was 0.55.

Higher NT-proBNP concentrations were associated with increased risk of HF hospitalization (HR 3.11 [2.53-3.82], p<0.001; C-statistics 0.85) and all-cause mortality (HR 2.49 [95% CI 2.04-3.03], p<0.001; C-statistics 0.82). These associations were also statistically significant in adjusted models.

Greater changes in NT-proBNP concentrations from the baseline visit to the follow-up visit (after mean 1,4 y) were associated with an increased risk of subsequent HF hospitalization (HR 1.73 [95%CI 1.03-2.90] p=0.04) and mortality (HR 1.71 [1.05-2.77], p= 0.03), respectively in the adjusted models, but not with subsequent incident VA (HR 1.00 [0.66-1.52], p=0.98).

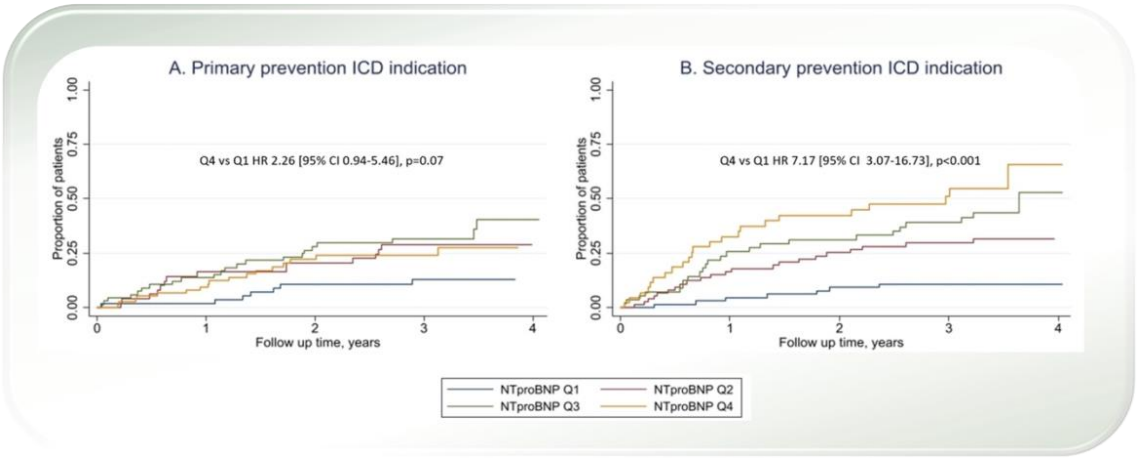

Figure 10 Association between baseline concentrations of NT-proBNP and time-to-first ventricular arrhythmia in patients with (A) primary prevention ICD indication and (B) secondary prevention ICD indication, stratified by quartiles of NT-proBNP. Also presented is the results of a Cox regression analysis for quartile 4 versus quartile 1. (Own figure)

5.3 Paper III

In **paper III,** we assessed the associations between concentrations of cTnT, GDF-15, IL-6, and CRP and incident VA in 489 patients treated with ICD. We demonstrated that higher concentrations of cTnT were associated with an increased risk of VA (HR 1.63 [95% CI 1.31-2.01] per log-unit, p<0.001; C-statistics 0.62), which also persisted after adjusting for age, sex, BMI, CAD, HF, LVEF and eGFR. The association between cTnT concentrations and incident VA was consistent in patients with primary and secondary ICD indication (p-for-interaction=0.25) (**Figure 11, 12**). GDF-15, IL-6 and CRP were not associated with the risk of VA (**Figure 12).**

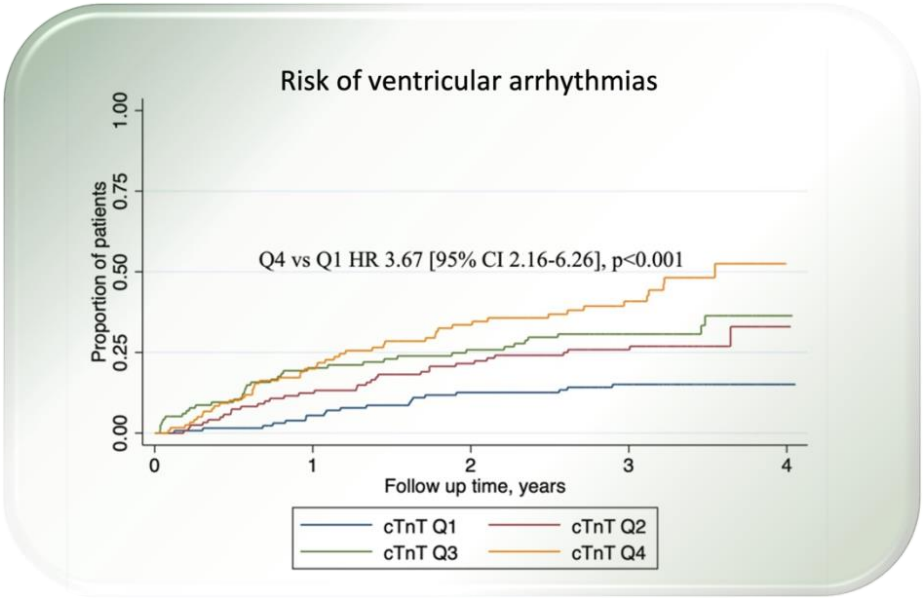

Figure 11 Survival analysis of time to ventricular arrhythmia, stratified by quartiles of cTnT. Also presented are the results from a Cox regression analysis for quartile 4 versus quartile 1.(Own figure)

Higher cTnT concentrations were associated with increased risk of hospitalization for HF, (HR 3.85 [2.88-5.14], p<0.001, C-statistics 0.80) and all-cause mortality (HR 4.77 [3.43-6.64], p<0.001, C-statistics 0.82) (**Figure 12),** which persisted in the adjusted models and was consistent across ICD indication. Changes in cTnT concentrations from the baseline visit to the follow-up visit (after mean 1.4 y) were not associated with subsequent VA (p= 0.66), HF hospitalization (p=0.58), or mortality; (p=0.90).

GDF-15, IL6 and CRP concentrations at baseline were all associated with increased risk of HF hospitalization with C-statistics 0.79, 0.70, and 0.62, respectively. **(Figure 12)**. We found no significant associations between changes in biomarker concentration during follow-up and HF hospitalization. GDF-15, IL-6 and CRP were also associated with increased risk of all-cause mortality (C-statistics 0.78, 0.75, and 0.67, respectively), which persisted in the adjusted models. Changes in GDF-15 concentrations (HR 2.66 (1.59-4.45), p<0.001), but not IL-6 or CRP, were associated with the risk of all-cause mortality.

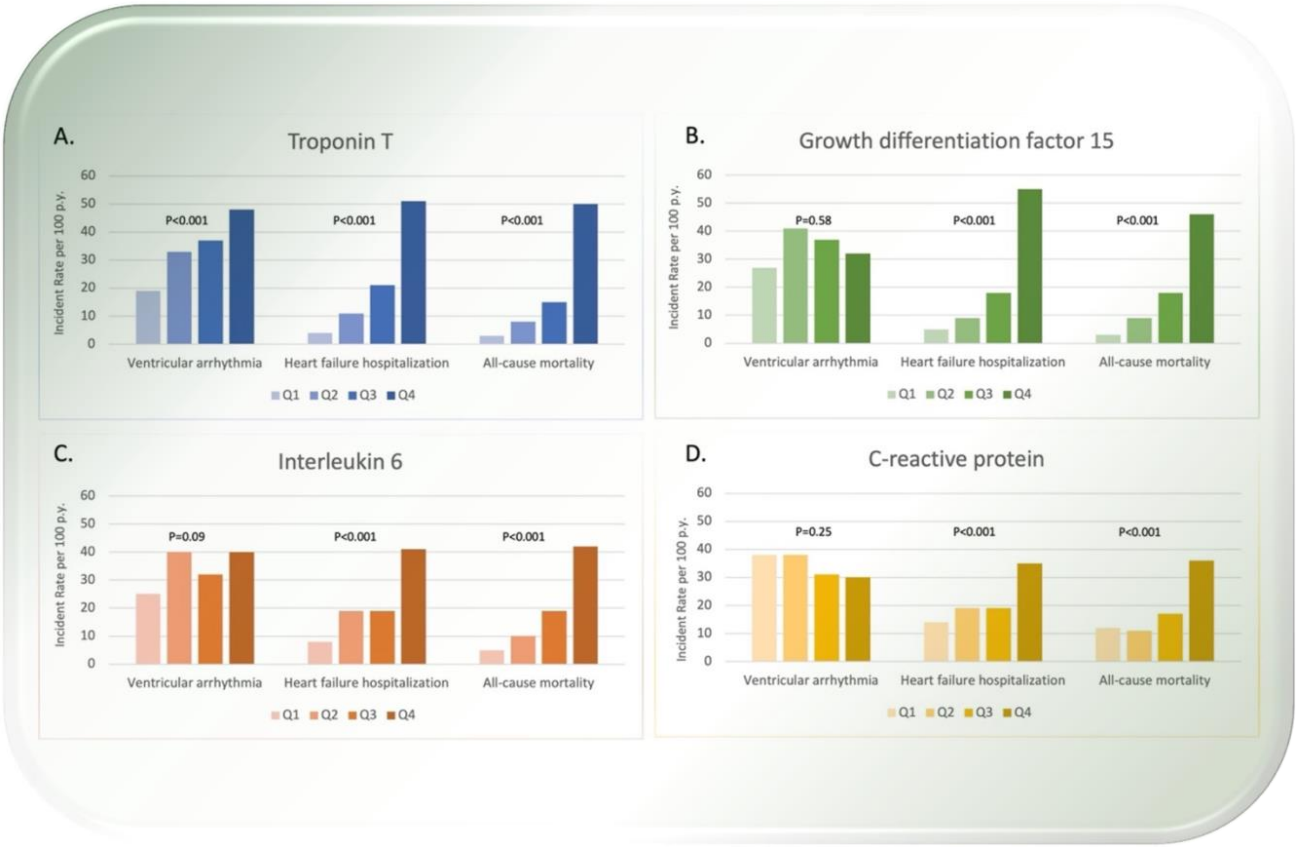

Figure 12 Incident rates of ventricular arrhythmia, heart failure hospitalization, and all-cause mortality, according to quartiles of baseline cardiac troponin T (A), growth differentiation factor-15 (B), interleukin 6 (C), and C-reactive protein (D) (*Own figure)*

6. Discussion of main findings

6.1 General findings

This thesis has three main findings: ***(1)*** the presence of fQRS on 12-lead ECG is associated with increased risk of VA during follow-up, ***(2)*** higher concentrations of NT-proBNP and cTnT, but not the inflammatory biomarkers GDF-15, IL-6, and CRP, are associated with increased risk of VA, and ***(3)*** higher concentrations of NT-proBNP, cTnT, GDF-15, IL-6, and CRP are all associated with increased risk of HF hospitalization and all-cause mortality in patients treated with ICD.

6.2 fQRS for VA risk prediction

Fragmentation of the QRS complex on ECG has been proposed to reflect altered cardiac depolarization caused by non-uniform activation of the myocardium. Myocardial fibrosis and scar may underlie non-uniform activation of the myocardium,[62,63,156] which is supported by the presence of regional perfusion defects during nuclear stress testing[62] and delayed gadolinium enhancement on cardiac magnetic resonance imaging in

patients with fQRS.[156] Recent ESC guidelines recognize fQRS as suggestive of underlying CAD in patients presenting with sustained VT.[3] This is supported also by our results as we found the presence of fQRS to be associated with established CAD and prior MI in the SMASH 1 Study. However, one should recognize that type, volume, and location of myocardial fibrosis differ according to the pathophysiological process. [157] Replacement fibrosis occurs after myocardial damage where collagen replaces injured cardiomyocytes, such as found after MI. [157,158] In reactive interstitial fibrosis there is no loss of cardiomyocytes, but rather an increased production and deposition of collagen in extracellular matrix as found in cardiac remodeling in patients with hypertensive heart disease or HF. [157,158] Infiltrative interstitial fibrosis is another, but less common type of fibrosis that is characterized by accumulation of glycolipids, such as in patients with Fabry`s disease. [158] Many patients have combinations of different types of fibrosis, as is the case for patients with cardiomyopathies.[157] Different types of fibrosis cause different conduction disturbances and may also lead to different ECG fragmentation.[159]

fQRS has been recognized as a risk factor for developing VA in patients with Brugada syndrome[160,161] and in patients with treated Tetralogy of Fallot.[160] Recent ESC guidelines have included fQRS in the risk stratification for the primary prevention of SCD in patients with treated Tetralogy of Fallot. [3] Prior studies have also demonstrated an association between fQRS and risk of VA in patients with CAD, dilated cardiomyopathy, HF, and in patients treated with ICD.[59,162,163] In agreement with previous studies, we found fQRS to be associated with a 3-fold increased risk of device-detected VA in a large heterogenous population treated with ICD irrespective of established risk factors, with a specificity of 94% for detecting VA during follow-up.

Prior studies of fractionation in ECG have proposed that heterogeneous and delayed conduction abnormalities may explain the fragmentation of ECG. [164,165] In our study, the presence of fQRS was associated with a history of VA (secondary prevention ICD indication), in addition to previous MI and older age. This observation supports the main finding of a link between fQRS and ventricular arrhythmogenicity.

Our objective was to investigate fQRS as the key ECG-variable of interest for VA-risk prediction, and this was prespecified in our analysis plan. Interestingly, other ECG parameters such as QRS duration, QRS axis, QTc, and heart rate were not associated with the presence of fQRS (among patients with native QRS). Moreover, fQRS was found superior for predicting VA to all other ECG variables that we analyzed, such as T-peak to T-end, QT interval and QRS duration. Finally, the association between fQRS and VA was independent of QRS duration and the presence of BBB. These findings suggest that the arrhythmic properties associated with fQRS are more likely due to heterogeneous ventricular depolarization, rather than homogenous conduction disturbances or conduction delay *per se.* However, the lack of electrophysiological examinations in our study limits our ability to determine the pathophysiological mechanisms behind fQRS in our patients.

The association between fQRS and VA persisted after adjusting for ICD indication. However, there was a significant interaction, suggesting that the association was stronger among patients with primary prevention ICD indication compared to patients with a secondary prevention ICD indication. Furthermore, among patients with primary prevention ICD, the association between fQRS and VA was *not* modified (no significant interaction) by the presence or absence of CAD, suggesting that it can also play a role in discriminating patients with non-ischemic HFrEF. Given the results from the DANISH study, this is a group where it is particularly important to risk stratify patients. Importantly, as this is a subgroup of a subgroup, the results for this specific group should be interpreted with caution and hopefully investigated in a future study of non-ischemic HFrEF.

Based on our findings in Paper I and previous work in the field, we propose that fQRS on ECG could be used as an additional tool to help distinguish patients at high risk of VA from those with a lower risk, particularly in the primary prevention setting. If these findings are replicated in prospective studies of non-ischemic HFrEF patients with uncertain indication for ICD treatment, fQRS assessment could be a useful tool for evaluating initiation or continuation of antiarrhythmic treatments and potentially even help identify patients are more likely to benefit from ICDt. [56] However, these findings need to be validated as our findings should only be considered hypothesis-generating.

6.3 NT-proBNP

NT-proBNP concentrations are elevated in HF and have been shown to be predictive of major cardiovascular events in HF patients. NT-proBNP concentrations also predict clinical outcomes in subjects from the general population and in patients with CAD. [72-74] We demonstrated that higher concentrations of NT-proBNP were associated with a worse prognosis in a heterogeneous group of patients treated with ICD. Thus, our findings extend those from prior studies, which have consistently demonstrated NT-proBNP concentrations to be reflective of myocardial wall stress, neurohormonal activation, aging, and reduced renal function. We found a history of HF, worse renal function, and older age to be independent predictors of higher concentrations of NT-proBNP in our study.

What was more novel, and the main finding from Paper II, was that higher baseline NT-proBNP concentrations were predictive of VA, with around a 4-fold increased risk among patients in the highest compared with the lowest quartile. Importantly, this association was independent of established risk factors, including the most frequently used risk stratifier; LVEF. Prior studies have demonstrated an association between higher concentrations of NT-proBNP and the risk of *clinically suspected SCD*, also irrespective of LVEF.[86] However, many of the previous studies have defined SCD as death presumed to be arrhythmic, and poorly discriminate arrhythmic from non-arrhythmic etiologies of SCD such as acute circulatory collapse without an identifiable triggering event [166] or non-cardiac cause for death such as pulmonary embolism, aortic rupture, and acute cerebral hemorrhage. [167] In our study we only included device-detected VA, and to our

knowledge, it is the biggest prospective study to study the association between NT-proBNP (or BNP) and device-detected VA, as well as having the longest follow-up.

The exact pathophysiological mechanism explaining the association between higher NT-proBNP concentrations and VA risk is unknown. However, myocardial stretch, which is thought to be the main mechanical stimulus for NT-proBNP release, is believed to trigger arrhythmia [166,168] through mechano-electric feedback. [44,169] Mechano-electric feedback refers to the electrophysiological changes caused by mechanical changes in the myocardium and is believed to contribute to the increased arrhythmic risk in diseases and pathological conditions with altered cardiac wall mechanics. [44,169] By affecting a sequence of stretch-activated currents, cell-cell gap junction currents and altering cell membrane capacitance, MEF can induce physiological and electrical heterogeneities in the heart, [169] which can potentially cause VA through several mechanisms triggering automaticity, triggered activity, and reentry. [44]

Our results demonstrated a stronger association between high NT-proBNP concentrations and risk of VA among patients with secondary ICD-indication, among whom the association persisted in the adjusted models, whereas it was non-significant for patients with primary ICD indication. This finding may seem counterintuitive as patients with a primary prevention ICD indication from severe HF have higher NT-proBNP concentrations. However, this may in fact be the explanation for the limited association between NT-proBNP and VA risk in these patients, as there are hemodynamic and neurohormonal mechanisms that primarily drive the NT-proBNP expression in HF. Pertinent to this point, NT-proBNP concentrations were strongly associated with secondary endpoints like HF hospitalization and all-cause mortality in our cohort, which reflects the strong, non-specific risk abilities of NT-proBNP in patients with ICD.

6.4 cTnT

In paper III we demonstrated that higher cTnT concentrations were associated with increased risk of VA, HF hospitalization, and all-cause mortality. Patients with cTnT levels in the highest quartiles had around 4-fold higher risk of VA compared with patients in the lowest quartile. This association was independent of established risk factors such as LVEF, previous MI, and renal function. Although higher concentrations of cTnT were predictive of future episodes of VA, they were also associated with a *lower* prevalence of previous VA. This is due to higher cTnT concentrations in patients with HF and primary ICD indication, compared to non-HF patients with ICD due to previously documented VA (secondary ICD indication). This is supported by analyses demonstrating no association between cTnT and previous VA when each of the groups are analyzed separately.

Few studies have focused on the association between cTnT and the risk of VA or SCD. In a large study from the US of 3089 older participants from the Cardiovascular Health Study, cTnT was demonstrated to be associated with the risk of SCD. [170] Similarly a large case-control study of 6 prospective cohorts with 565 cases of clinically diagnosed SCD

demonstrated a significant association between higher concentrations of cTnI and increased risk of SCD. [171] Similar associations have been reported in patients with HF. In the TOPCAT trial of patients with HFpEF there was a significant association between higher cTnI levels and the risk of SCD (C-statistics 0.75), with 64% of clinically suspected SCD cases among patients with cTnI levels in the highest quartile. [172] However, the association between cTn and device-detected VA has not been analyzed and to our knowledge, our study is the first to address this.

Although the pathophysiological mechanism explaining the association between higher cTnT levels and risk of VA is unknown, cTnT is known to reflect mechanical myocardial damage. One could hypothesize that ongoing myocardial injury processes over time and subclinical fibrosis, reflected by higher levels of cTnT can act as substrates for VA and potentially SCD. CAD is frequently associated with SCD,[30] possibly due to myocardial injury or hypoperfusion of myocardial tissue, which are recognized triggers of ventricular arrhythmic events. [173] Interestingly, we found the association between cTnT concentrations and the risk of VA to be independent of the presence of ischemic etiology, suggesting that pathophysiological mechanisms besides myocardial ischemia account for the prognostic ability of cTnT to predict incident VA. Higher cTnT levels have been described in patients with both acute and stable chronic HF, as well as in patients with cardiomyopathy including hypertrophic- and dilated CM and have been illustrated to be a marker of poor prognosis even at low concentrations. [174-177] These findings support the role of cTnT as a marker for progressive cardiac remodeling and could be indicative of ongoing myocyte injury, hypertrophy, and increased fibrosis, [178,179] all of which are potent arrhythmogenic triggers. [159,168,180] Regardless of the mechanism, our results demonstrate a role for cTnT measurements to identify individuals at high risk of VA.

cTnT has consistently been demonstrated to be a predictor of HF hospitalization and mortality in the general population,[108,181] among patients with CAD,[112] and patients with HF.[172] In agreement with previous studies, we demonstrated that higher concentrations of cTnT were associated with increased risk of hospitalization for HF and all-cause mortality in ICD patients. This suggest that cTnT seems to be strongly associated with indices of HF and conventional risk factors for mortality. Indeed, higher cTnT concentrations in our study were associated with lower LVEF, higher NYHA functional class, and established diagnosis of HF. Additionally, higher cTnT concentrations were associated with older age, worse renal function, and several comorbidities such as DM, CAD, cardiomyopathy, and previous AMI, all of which contribute to the general risk of HF and mortality. Notably, the association between cTnT and each of the outcomes was present also after adjusting for these factors. The pathophysiological mechanism behind these associations is most likely a result of complex progressive cardiac remodeling due to chronic myocardial injury. Other mechanism may be subtle, progressive subendocardial ischemia or necrosis, coronary microvascular dysfunction, and increased oxygen demand. [182-184].

6.5 GDF-15, IL-6, and CRP

IL-6, CRP and particularly GDF-15, are strong prognostic markers with respect to major cardiovascular events and mortality. [123,141] GDF-15 has consistently, and in multiple clinical settings, been demonstrated to be a stronger prognostic marker than IL-6 and CRP. [128,130,185] We extend these findings to patients treated with ICD, where all three markers were associated with HF hospitalization and all-cause death. The discriminatory performance was quite strong for GDF-15 (C-statistics 0.79 and 0.78, respectively), intermediate for IL-6 (C-statistics 0.70 and 0.75, respectively) and modest for CRP (C-statistics 0.62 and 0.67, respectively). Furthermore, all three biomarkers added incremental prognostic information to that of cTnT for these outcomes.

Inflammation plays a critical role the development and progression of HF. GDF-15, IL-6, and CRP are believed to be secreted in response to various HF-related stimuli, such as cardiac remodeling, pressure overload, and myocardial ischemia.[143,186-188] Furthermore, concentrations of circulating GDF-15, IL-6, and CRP are believed to reflect the severity of inflammation, tissue damage, and to correlate with many conventional cardiovascular risk factors, such as age, smoking, DM, hypertension, and stress. [147,189-191] These biomarkers have been found associated with endothelial dysfunction and oxidative stress and, in addition proposed to be involved in cell survival and apoptosis regulation. [192-195] Thus, the prognostic value of these biomarkers in patients treated with ICD is not surprising and in agreement with prior HF studies.[141,142,196]

Interestingly, none of these inflammatory biomarkers were associated with the risk of incident VA. This is in agreement with a retrospective case-control study of patients with cardiomyopathy treated with ICD for primary prevention where GDF-15 concentrations did not associate with incident VA[197], but contrast studies reporting an association between high GDF-15 and IL-6 concentrations and risk of clinically adjudicated SCD. [198-200] However, these later studies had limitations with small sample sizes[199] and clinically defined cases of SCD as the endpoint[200]; and also included SCD events in the acute setting following MI.[198] These differences may explain the divergent results from device-detected VA in a cohort of patients with ICD from the outpatient clinic. Data from the Multi-Ethnic Study of Atherosclerosis with subjects free from CVD demonstrated no association between GDF-15 concentrations and subclinical cardiac fibrosis (replacement fibrosis and interstitial fibrosis), as accessed by cardiac magnetic resonance imaging and late gadolinium enhancement. In contrast, cTnT concentrations were associated with cardiac fibrosis in the same study, which supports that a cardiac-specific marker will reflect cardiac fibrosis better than an unspecific biomarker like GDF-15. [201] This difference may therefore also explain the stronger association between cTnT and VA compared to GDF-15 and VA in our study.

Still, there is a need for additional studies to better understand the role of these biomarkers in disease pathogenesis and risk stratification in ICD patients.

7. Methodological considerations

7.1 Study design and population

The SMASH 1 Study is a multicenter, observational clinical study. Patients were recruited from the outpatient clinics at the two study sites, AUH and SUH, both of which are teaching hospitals with AUH located in the South-Eastern part of Norway and SUH located on the West coast. We found consistent results between the two study sites. Nonetheless, as Norway has a homogenous, primarily Caucasian population, our results may not be valid in other regions of the world.

The main aim of the study was to study the associations between different biomarkers and the risk of incident VA. Since the study was prospective in design, it was well suited to explore the main research aims of the current thesis. By measuring events in a temporal sequence, where the exposure proceeds the outcome, the design of the SMASH 1 Study allows us to prospectively establish the associations between the studied biomarkers and primary and secondary endpoints. However, the observational nature of the study does not permit us to study causation, but rather associations between the biomarkers and the endpoints. Studies of causality will require other designs, like the use of Mendelian randomization studies to assess the association between the variance of genes known to express the proteins measured in this study and VA risk could provide insights on causality.

Our study is prone to different forms of bias, which are important to address. *Selection bias* occurs if the patient selection process leads to an inaccurate representation of the population of interest. Compared to retrospective studies, our prospective study is less prone to selection bias as the outcomes have yet to occur when the patient is included. Still, as we did not include patients with conditions that could impair their ability to participate in the study, such as short life-expectancy, we likely recruited a "healthier" cohort compared to the real-world population of ICD patients. We believe, however, that this is a reasonable exclusion criterion to minimize the number of losses to follow-up. Additionally, our cohort is prone to *gender bias*, as it comprises mainly of male patients (83%). However, this typically represents the gender distribution of patients treated with ICD and we randomly screened patients eligible for participation in the SMASH 1 Study, irrespective of sex. During data collection, patients were asked to provide information about previous medical history and functional class using a structured interview. This design could potentially make the study prone to *recall bias*. That would for example occur if patients would over- or underreport their medical history or report their functional class differently from their actual functional class. Still, such biases are more of a concern with retrospective studies where participants with events often are more engaged in the project and therefore answer questions differently compared to participants without events. Hence, we believe information- and response bias are less of a problem with our prospective data collection in the SMASH 1 Study. To minimize the risk of recall bias we also conducted a thorough review of the electronic health records

to verify and supplement information provided by the patients. Finally, our study is prone to *confounding bias* as many risk factors are associated with both the exposure variables investigated (fQRS and circulating biomarkers) and associated with the risk of developing VA, HF hospitalization, and death. For instance, eGFR is associated with concentrations of circulating biomarkers due to renal excretion and shared risk factors, and eGFR is also a strong predictor of cardiovascular risk. To reduce the risk of confounding bias we adjusted for a range of potential confounders that were selected *a priori* based on previous studies and current knowledge in the field. Still, we acknowledge that there is a chance of residual confounding.

7.2 ECG analyses

All ECGs in the SMASH study were analyzed by two independent adjudicators from the two study sites to reduce the risk of observer bias for paper I. All ECGs were interpreted for fQRS by two independent adjudicators, and disagreement on the adjudication was solved by consensus, involving a third adjudicator. In this process, the consensus rate was 99% between the two reviewers for fQRS assessment and we therefore believe the bias in ECG adjudication was limited in our study. We used paper-based ECGs in the SMASH 1 Study, which imposes restrictions on precise analysis, particularly those of minor segments and amplitudes. Since that time, there has been a gradual transition to a digitalization of ECG recordings, which will allow for additional features to optimize ECG analysis, such as zoom options and filter edition. Further, the use of signal-averaged ECG technique can reduce noise, which allows better visualization of low-amplitude signals and high-resolution recording to allow higher analytical precision, including diagnosing notches in the QRS complex.

7.3 Biomarker sampling

In the biomarker analyses for papers II and III, a potential reason for bias can be the biological and analytical variability of the studied biomarkers. To overcome this bias, we performed all analyses in the same laboratory, the core laboratory at AUH, and in one batch with the same instruments and assays. Protein biomarkers are also prone to degradation upon freezing and long-term storage. NT-proBNP has been demonstrated to be stable when stored at different storage conditions, [202,203] including for at least 1 year when stored at -80°C. [204] NT-proBNP also seems to have minor variability in concentrations after repeated freeze-thaw cycles, including only 1.6% in mean percentage reduction in concentration after five freeze-thaw cycles. [204] For cTnT, a study demonstrated minor analytical variation after 12 months of storage and two freeze-thaw cycles. [205] Another study reported a mean degradation of 0.36 ng/L per year when stored over a period of 36 months and later only modest degradation after an average of 8 years storage at -80°C. We believe these studies support excellent stability of cTnT at -80°C and that analytical variation from one freeze-thaw cycle should be considered minor. [206] Previous evaluations of GDF-15 have shown that GDF-15 concentration are stable at room temperature for 48 hours and are resistant to 4 freeze-thaw cycles, [120] while CRP was shown to be stable for up to 34 months when stored frozen at -20°C.[207] IL-6 has been shown to be stable throughout multiple freeze cycles, but it was shown to

degrade in up to 50% of measured baseline values within 2-3 years of storage. [208] Generally for cytokines it is recommended to store the samples at -80°C or below to limit degradation for long time storage, and as for the remaining biomarkers, it is advised not to perform repeated freeze-thaw cycles to further limit degradation and ensure stability. To ensure optimal stability of our biomarkers and limit degradation due to storage, we stored our samples at -80°C and did not perform repeated freeze-thaw-cycle as only samples that had not previously been thawed were used for the current project.

7.4 ICD-programming

Giving the fact that we did not have standardized ICD-programming in our study protocol, there is a potential for bias. However, physicians who programmed and interrogated the ICD recordings were all blinded to outcome data, to ECG results and to biomarker analyses, making it unlikely that this variability should markedly influence our results. It can also be considered a strength that the ICD programming was according to best clinical practice for each individual patient, which increases the external validity.

7.5 Imaging modalities

In our study, we had limited imaging modalities for assessing cardiac function and structure. We obtained information from the patient's electronic medical records on the most recent measured LVEF, based on either a clinically recorded echocardiographic examination or a cardiac magnetic resonance imaging examination. Although the majority had LVEF measured by echocardiograms, the difference in modality may introduce bias. The time between these prior imaging examinations and study inclusion will differ for individual study participants. The lack of standardized imaging is a limitation to our study and reduces our ability to detect cardiac pathobiology responsible for the identified associations between fQRS and biomarkers and validated ventricular arrhythmic events and different clinical outcomes. Mechanical dispersion by myocardial strain imaging could be of special interest, as mechanical dispersion is associated with VA risk and also associated with concentrations of both cTn and NT-proBNP. [209-211]

7.6 Clinical endpoints

The majority of studies in this field use SCD based on clinically suspected events as the outcome, which potentially can include also non-cardiac and cardiac death not related to arrhythmia. The primary endpoint of this thesis was therefore more precisely defined as device-documented events due to VA events, more specifically sustained VT >100 b.p.m over 30 seconds, VF, or appropriate ICD treatment for VA.

Information regarding hospitalization for HF and death were obtained from electronic medical journal and reviewed by a physician. Thus, the reason for hospitalization was not only based on registered diagnosis at the time of discharge but also adjudicated events reviewed by a physician and adjudicated based on the totality of clinical information. We believe this approach will make our clinical endpoints accurate. Information concerning patients dying during study follow-up was collected from the Norwegian Death Registry, and the number of all-cause deaths are therefore complete.

We adjudicated the cause of death based on the electronic health record for the non-survivors who had this available. Importantly, not all patients have their ICD interrogated post-mortem, so we may have missed some cases of death from VA that was not successfully treated by the ICD.

7.7 Statistical aspects

Statistical analyses applied in all 3 papers were based on conventional analytical methods. All statistical tests were used as recommended depending on the distribution of the data studied and the assumptions necessary for each test.

In all three papers in this thesis, covariates were chosen *a priori* based on established and suspected risk factors of the outcomes. We also included covariates known to influence the studied biomarkers. [212] Age, gender, BMI, eGFR, HF, LVEF, and CAD are risk factors associated with the risk of SCD, HF decompensation, and mortality. [213-215] Additionally, many of these variables are also associated with circulating cardiovascular biomarker concentrations. For instance, HF and LVEF correlate positively and strongly with NT-proBNP concentrations, and BMI correlates inversely with NT-proBNP concentrations.[216] In Paper I, where we studied the association between fQRS on ECG and risk of VA, we also adjusted for ECG parameters that potentially can be cofounders for fQRS and the outcome of VA, like QRS duration, QRS axis, presence of Q-wave, and BBB. In addition to the covariates mentioned above, hypertension, DM, levels of VES burden on ECG, and potentially also other variables could represent potential confounders. [213] However, due to moderate sample size and a modest number of events, we did not include all possible risk factors as covariates in the multivariable models to avoid the risk of over-fitting of the models, which would have reduced the precision of the effect estimates and introduce type II errors. [217]

In paper II, we stratified our analyses based on the ICD indication (primary or secondary prevention), and in paper III we stratified for ischemic heart disease etiology (yes/no). These analyses were generated post-hoc and the results should therefore be considered hypothesis-generating.

The SMASH 1 Study was conducted as a multicenter study with patients included from two centers. Optimally, our results should have been validated in an external cohort. Prior to the initiation of the SMASH 1 Study we considered the possibility of dividing the cohort into a derivation cohort (AUH cohort) and a validation cohort (SUH cohort). However, due to limited statistical power, we decided to merge data from the two centers and therefore we lack a validation cohort for this work. We performed interaction analyses based on study center and did not find any differences between the two teaching hospitals including participants in the SMASH 1 Study.

We used relative changes in biomarker concentrations from baseline to follow-up visit to assess associations between temporal changes in biomarker concentrations and endpoints in papers II and III. Relative change is calculated as ratios, i.e., biomarker

concentration at follow-up divided by baseline concentrations, which will provide the proportional change in concentrations from baseline to follow-up. Alternatively, one could have calculated absolute change, which is biomarker concentration at follow-up visit minus concentrations at baseline. However, due to the non-normal distribution of biomarker concentrations in our study and the risk of marked influence from patients with high baseline values to absolute changes, we chose to use relative change in biomarker concentrations for these analyses.

To ensure a reliable answer to the study hypothesis, estimating sample size is important when planning a clinical study. If the study includes a low number of participants, there is a risk for type II error, which means that the study is not able to statistically demonstrate a true difference (in the overall population) between the groups of the study cohort (i.e. failing to reject a false null hypothesis of no assumed difference between the groups). The statistical power calculation for the SMASH 1 Study was performed based on the hypothesis of identifying a biomarker that predicted VA and SCD. With assumption of 12 months follow-up time, 33% of the patients were expected to be in the high biomarker group and 67% in the low biomarker group and annual incidence of VA was considered 20% in the high-biomarker group and 10% in the low-biomarker group. Accordingly, with alpha = 0.05 (type I error rate), the study was estimated to need 454 patients to have a statistical power of 90% to detect a doubling in risk between the two groups, which was considered clinically relevant. With a maximum drop-out rate of 10%, we therefore aimed to enroll 500 patients in the SMASH 1 Study.

8. Ethical considerations and funding

The SMASH 1 Study was approved by the Regional Ethics Committees with case reference number 2015/2080. All included patients in this study signed a written informed consent prior to study commencement and the study was performed in accordance with the Helsinki Declaration. Personal data was anonymized using an assigned study ID and data handling was done according to the regulation of the local Data Protection Official and stored in secure data areas.

An ethical aspect of this study are examinations that participants must undergo as part of the study, without it having any treatment consequences for them. In our case, this primarily relates to the collection of blood samples, which requires a venipuncture with the complications that may follow, such as hematoma, local infections, bleeding, vasovagal syncope, edema, and local thrombus generation. To limit these complications, all venipunctures were performed by trained study nurses. Participants also underwent ECG-recording twice, with the chance for skin irritations and allergies to electrode patches used during ECG examinations. However, none of these complications are considered serious and we did not experience any serious complication during study execution.

Funding of the SMASH 1 Study was provided by the Research Council of Norway and by grants from Akershus University Hospital and the South-Eastern Norway Regional Health Authority. Assays for the analyses of NT-proBNP, cTnT, IL-6, CRP and GDF-15 were provided by Roche free of charge.

9. Conclusion & Implications /future perspective

In this thesis we found biomarkers that can aid in assessing the risk of incident VA and clinical outcomes in patients treated with ICD.

More specifically, we demonstrate that the presence of fQRS on ECG and higher baseline concentrations of NT-proBNP and cTnT were associated with an increased risk of incident VA. For NT-proBNP concentrations, the association with VA were present irrespective of established risk factors and LVEF and appeared to be stronger for patients with secondary prevention ICD indication. High baseline concentrations of cTnT were also associated with increased risk of incident VA, independent of established risk factors and prior ischemic heart disease. High concentrations of NT-proBNP, cTnT, GDF-15, IL-6 and CRP on study inclusion were all associated with increased risk of HF hospitalizations and all-cause death during follow-up. For both NT-proBNP and cTnT, the risk discrimination was stronger for HF-hospitalization and all-cause death, than for VA.

Clinical implications and Future perspective

ICD remains the reference treatment to prevent future VA events in patients at risk. As discussed in this thesis, predicting VA remains a long-standing clinical challenge. Hence novel markers could be of value to improve risk assessment for VA. However, no single biomarker has yet been shown to have sufficient precision in predicting VA and SCD. Therefore, using panels of different risk markers, including ECG parameters and circulating biomarkers in addition to clinical and cardiac assessment may be a future strategy to risk stratify patients for incident VA and SCD. In this work, we report several markers that seem to have the potential to help in the stratifications for VA risk. Our results suggest that fQRS, NT-proBNP and cTnT, have the potential to aid in assessing patients at risk of VA and that all studied biomarkers can additionally identify patients at higher risk of HF hospitalization and all-cause mortality.

Biomarkers that predict VA are particularly warranted in primary prevention. fQRS was more strongly associated with VA risk in patients with a primary prevention ICD indication, in comparison with secondary prevention indication. cTnT was predictive in both groups, while NT-proBNP was not significantly associated with VA risk in primary prevention. As mentioned earlier in this thesis, radical improvements in HF pharmacotherapy have reduced the incidents of SCD, and the role of ICD is more in question in contemporary care. Particularly, as illustrated by the DANISH trial, ICD treatment seems to be less efficient in patients with primary ICD indications due to non-ischemic HF. [56] In these patients, and generally among patients evaluated for ICD for primary prevention of SCD, fQRS and measurements of cTnT seems to add prognostic

information with respect to VAs and mortality. However, due to the observational nature of this study and adherent limitations, this hypothesis should be evaluated and validated in future studies before clinical use. Additionally, there is a need to better understand the pathophysiological mechanisms explaining the link between these markers and VA risk. Ideally, the strategy would be tested in a prospective randomized clinical trial where patients considered for ICD implementation are randomized to either a biomarker-guided evaluation for VA risk assessment or standard care according to current guidelines.

In patients with a secondary prevention indication for ICD, our data suggest that measurements of NT-proBNP and cTnT can aid in optimizing the VA risk assessment. The association between the concentrations of these cardiac biomarkers and the risk of VA was independent of age, sex, LVEF, renal function and comorbidities, including HF. In fact, NT-proBNP was particularly predictive of VA risk among non-HF patients, as the association was stronger in patients with secondary ICD indication than patients with primary ICD indication. Assessment of these biomarkers, which are widely used in clinical practice for other purposes, can be easily implemented in VA risk prediction. However, further studies are needed to validate our findings before being used clinically e.g., aid clinicians in considering the need for antiarrhythmic medications in patients with an ICD implanted for secondary prevention.

All the six circulating biomarkers studied in this thesis provide prognostic information with respect to HF hospitalization and death. Ongoing cardiac stress and systemic inflammation indicate a higher risk of HF hospitalization and mortality which may impact the aggressiveness of treatment and intensification on monitoring. [166] Not surprisingly, many of the biomarkers correlated significantly. Although we demonstrated incremental prognostic value by the inflammatory biomarkers on top of the cardiac specific biomarkers, we would not necessarily recommend routine measurements of these solely for the evaluation of HF hospitalization and mortality risk. However, they could serve as a tool in specific cases and should alert physicians of an inflammatory phenotype and increased cardiovascular risk. However, future studies are needed to evaluate whether treatment aiming to lower concentrations of inflammatory biomarkers would help reverse their increased cardiovascular risk. If used clinically, physicians should be aware of conditions that could falsely affect the results, such as an active infection, cancer, or autoimmune diseases.

It is important to underline that many of the pathophysiological mechanisms for the associations between these markers and the risk of the studied endpoints are not well known and one should therefore be cautious of the limitations of these recommendations. Further studies are needed to better understand the pathophysiological mechanisms behind the demonstrated associations, and ideally a clinical trial randomizing physicians to either get the results from these biomarkers or not is ultimately needed to prove that measuring the biomarkers improves outcomes.

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11. Articles I-III

I

Paper I

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ORIGINAL ARTICLE

WILEY

QRS fragmentation is associated with increased risk of ventricular arrhythmias in high-risk patients; Data from the SMASH 1 Study

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Abstract

Introduction: QRS fragmentation (fQRS), defined as the presence of additional spikes within the QRS complex, has been associated with myocardial conduction abnormalities and arrhythmogenicity.

Objective: We aimed to assess whether fQRS is associated with incident ventricular arrhythmias (VA) in high-risk patients treated with implantable cardioverter-defibrillator (ICD) for primary and secondary prevention.

Methods: In a prospective observational multicenter study, we included 495 patients treated with ICD. fQRS was analyzed according to previously validated criteria, by two physicians blinded for outcome data. Incident VA were obtained from ICD recordings.

Results: ECG recordings interpretable for fQRS were available in 459 patients (93%), aged 66 ± 12 years with left ventricular ejection fraction 40% ± 13%. fQRS was present in 52 patients (11%) with comparable baseline characteristics to patients without fQRS, except higher age, higher prevalence of coronary artery disease (CAD), lower prevalence of cardiomyopathy, and more frequently a secondary prevention ICD indication. Among patients with native QRS, those with fQRS had similar QRS duration and axis to those without fQRS. During 3.1 ± 0.7 years follow-up, 126 patients (28%) had ≥1 VA . fQRS was associated with increased risk of VA (HR 3.41 [95% CI 2.27–5.13], $p < .001$), which persisted after adjusting for age, gender, sex, BMI, CAD, heart failure, renal function, ICD indication, QRS duration, QRS axis, Q waves, and bundle branch block. fQRS was more strongly associated with VA in patients with a primary (HR 6.05 [95% CI 3.16–11.60]) versus secondary (HR 2.39 [95% CI 1.41–4.04]) ICD indication (p-for-interaction = .047).

Conclusions: fQRS is associated with threefold increased risk of VA in high-risk patients, independent of established risk factors.

KEYWORDS
cardiac arrest, implantable cardioverter-defibrillator, QRS fragmentation, risk prediction, ventricular arrhythmia

Study Registration

SMASH 1 Study; ClinicalTrails.gov Identifier: NCT02864771.

1 | INTRODUCTION

Sudden cardiac death (SCD) is a major global health challenge. Currently, the best-known predictor of SCD is either the presence of severe heart failure (HF) or an episode of cardiac arrest. Hence, guidelines recommend treatment with implantable cardioverter-defibrillator (ICD) as primary prevention in patients with symptomatic HF (New York Heart Association [NYHA] classification II-III) and left ventricular ejection fraction (LVEF) ≤35% (Priori et al., 2015). Patients with documented ventricular fibrillation (VF) or sustained ventricular tachycardia (VT) with hemodynamic consequence are also recommended for treatment with ICD (secondary prevention; Priori et al., 2015). However, several studies have found this approach to poorly discriminate patients at high risk of SCD (Buxton, 2003; Pascale et al., 2009).

Ventricular arrhythmias (VA) account for the majority of SCD cases with coronary artery disease (CAD) as the most frequent etiology (Koplan & Stevenson, 2009; Zipes & Wellens, 1998). However, the total burden of less common underlying conditions and arrhythmogenic mechanisms make the prediction of VA challenging. This underlines the need for strategies to identify patients at high risk of VA and SCD. Some electrocardiography (ECG) parameters have been associated with risk of VA, but the results are diverging.

The QRS complex represents ventricular depolarization, and disturbances in the depolarization may cause arrhythmias. QRS fragmentation (fQRS) is a morphological change in the QRS complex and is recognized as additional fractionation or notches within the QRS complex. Presence of fQRS suggests disturbed cardiac depolarization due to myocardial conduction abnormality caused by a non-uniform ventricular activation usually due to myocardial scarring (Das et al., 2006). fQRS has been identified as predictor of VA in patients with Brugada syndrome (Morita et al., 2008) and in patients with CAD and dilated cardiomyopathy (Das et al., 2010; Ratheendran et al., 2020). Accordingly, in the current study we aimed to determine whether fQRS is associated with risk of VA in a heterogeneous population with ICD. We hypothesized that the presence of fQRS in a standard 12-lead ECG is independently associated with risk of VA in patients treated with ICD.

2 | METHODS

2.1 | Study design and study population

The SMASH 1 Study (Scandinavian Multicenter study to Advance risk Stratification in Heart disease—ventricular arrhythmias; NCT#02864771) is a multicenter, observational, prospective study aiming to help identify predictors of VA in patients treated with ICD. Eligible patients were consecutively included during their regular outpatient follow-up visits for ICD control between May 2016 and March 2018 at Akershus University Hospital or Stavanger University Hospital in Norway. All patients treated with ICD, aged ≥18 years, were eligible for enrollment. Inclusion and exclusion criteria are presented in Figure 1. All patients provided informed written consent, and the study was approved by the Regional Ethics Committee (2015/2080) and the local Data Protection Officers at the institutions.

At the baseline visit, patients underwent a standardized interview to obtain information regarding medical history, symptoms, and medication use. Diabetes mellitus (DM), HF, cardiomyopathy, and CAD were defined by review of medical records and patient interviews. Measurements of heart rate and blood pressure (three measurements, where the average of the 2nd and 3rd measurement was used) were performed after 5-min rest. Standard 10-s 12-lead ECG recording was obtained upon inclusion using Mortara 350/380 (filter 150 Hz, AC filter 50 Hz, 50 mm/s, 10 mm/mV) or Schiller AT-110 (filter 150 Hz, AC Filter 50 Hz, 50 mm/s, 10 mm/mv). Blood samples were collected by venipuncture and analyzed by the core laboratory at each hospital.

2.2 | Outcome measures

The primary outcome of the SMASH 1 Study was defined a priori as episodes of ventricular fibrillation (VF) or ventricular tachycardia (VT) that were sustained (>100 beats per minute >30 s) or resulting in appropriately delivered ICD therapy (electrical shock or anti-tachycardia pacing [ATP]). Secondary outcome was defined as death from any cause. Arrhythmic events were obtained from ICD interrogations and/or hospital records during follow-up and include ICD-recorded (monitored and treated events) and clinically recorded events (including sustained VT episodes outside programmed monitor/treatment zones) and were conducted by experienced cardiac electrophysiologists. Study investigators reviewed the ICD recordings and reports in the electronic healthcare record and performed validation to ensure that only real events and appropriate ICD therapies were included as outcomes in this analysis. Adjudicators did not have knowledge of fQRS in the baseline ECG. In a sensitivity analysis accounting for death as a competing risk, we combined incident VA or appropriate ICD therapy with all-cause death. Clinical events were recorded by reports in the electronic healthcare records and by obtaining data from the Norwegian Cause of Death Registry (follow-up until 01.09.2020).

2.3 | ECG criteria for fQRS

Adjudication of fQRS on ECG was based on the following criteria as defined by Das et al. (2006), Das et al. (2008), Das et al. (2010): fQRS was defined as the presence of an additional R wave (R′), the

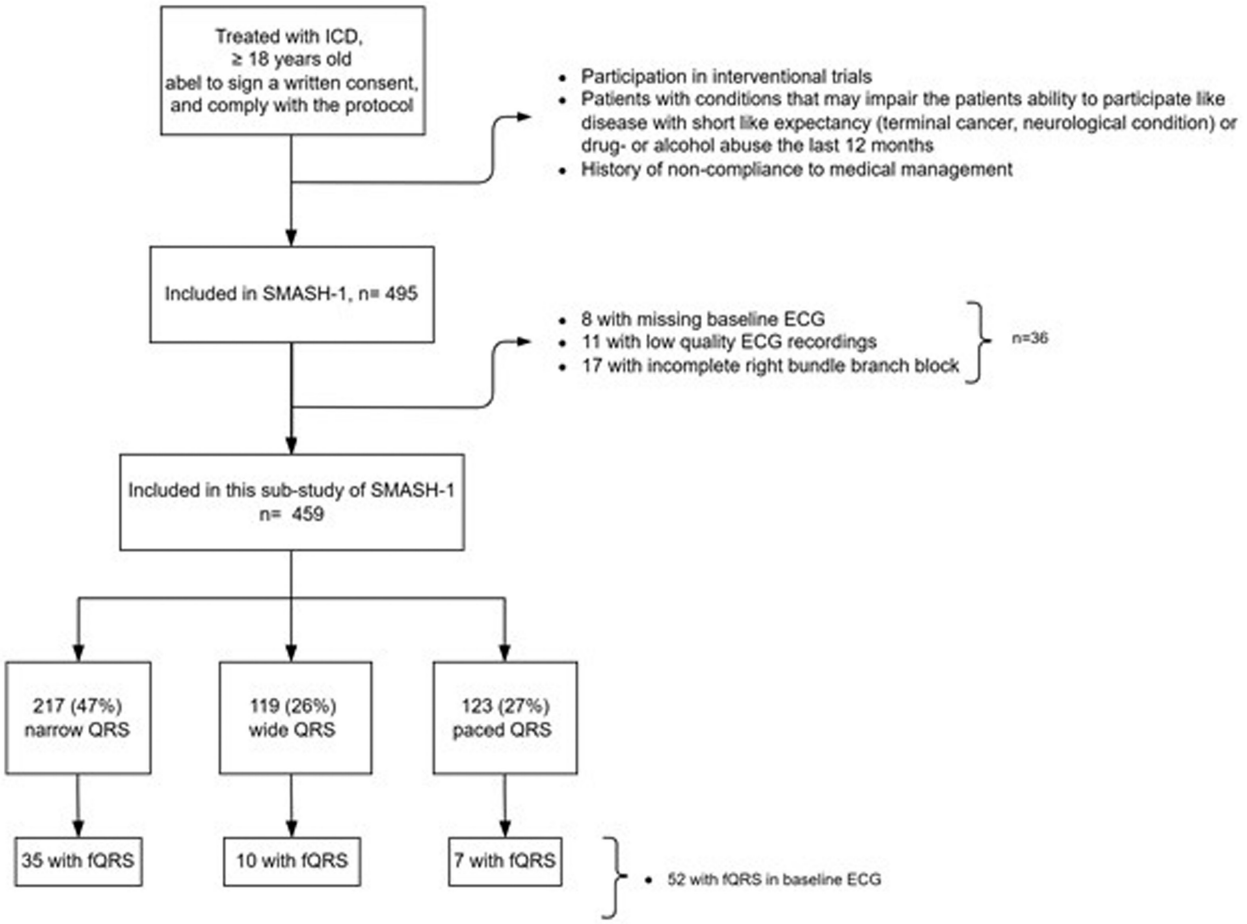

FIGURE 1 Flow diagram of the SMASH 1 Study

presence of >1 R′ (fragmentation) or notching in the downslope of the S wave (Figure 2a**)**. For ECGs with wide QRS complexes (>120 ms), including bundle branch block (BBB), fQRS was defined as various RSR′ patterns, with >2 R waves or >2 notches in the R wave or S wave (Figure 2b**)**. In case of paced QRS, fQRS was defined as the presence of >2 R′ or >2 notches in the S waves. fQRS was only classified if observed in two contiguous leads: Lateral leads (I, aVL, and V6) corresponding to left circumflex artery territory, anterior leads (V1–V5) corresponding to the left anterior descending artery territory or inferior leads (II, III, and aVF) corresponding to right coronary artery territory (Das et al., 2006). Patients with incomplete right bundle branch block (iRBBB) were excluded, as there is risk of over-interpretation of fQRS in these ECGs (Das et al., 2006; Das et al., 2008).

Two independent experienced physicians evaluated all ECGs blinded to outcome and adjudicated the presence of fQRS. If the two reviewers disagreed on the adjudication, this was solved by consensus, involving a third adjudicator if needed.

2.4 | Statistical analysis

Baseline characteristics are expressed as N (%) for categorical variables, median [Q1, Q3] for skewed continuous variables, and mean ± SD for normally distributed continuous variables. Patients with and without fQRS in baseline ECG were compared using ANOVA or *t*-test for continuous variables and Chi-square test for categorical variables. To assess independent predictors of fQRS, we performed a multivariable logistic regression analysis with fQRS as the dependent variable and age, sex, body mass index (BMI), CAD, HF, estimated glomerular filtration rate (eGFR), and ICD indication

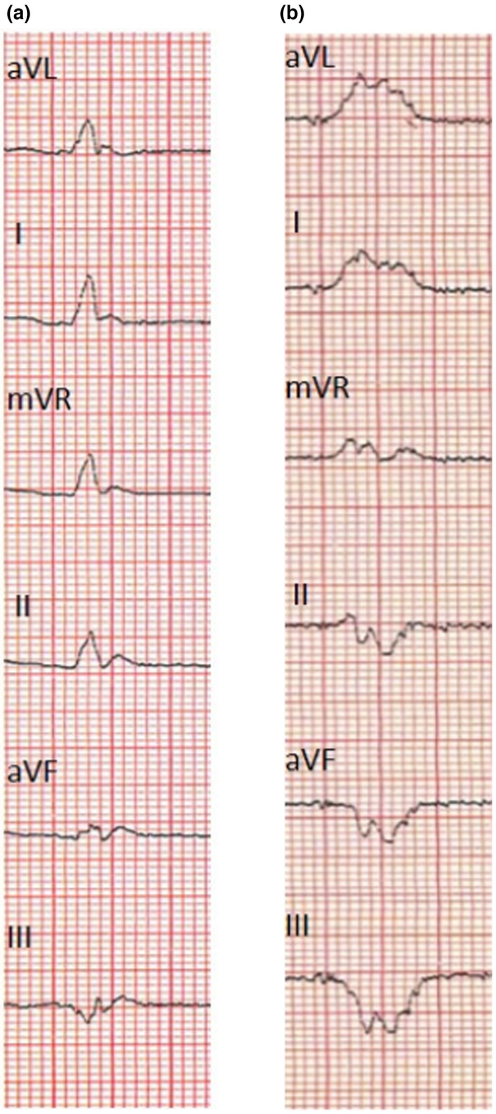

FIGURE 2 Example of fragmented QRS in a patient with narrow QRS (panel a) and wide QRS (panel b), with paper speed 50 mm/s. (a) Patient with QRS fragmentation in narrow QRS in both lateral and inferior wall (i.e., additional R wave). (b) Patient with QRS fragmentation in wide QRS in lateral wall (i.e., >2 notches in R waves) and also in inferior wall to some extent (i.e., >2 notches in S waves).

as independent variables. In patients with native QRS (without ventricular PM rhythm) on the baseline ECG, we performed an extended multivariable regression analysis also including baseline ECG parameters: QRS duration, QRS axis, presence of Q wave, and BBB. We examined the association between the presence of fQRS on baseline ECG and time to the first event of incident ventricular arrhythmias using Cox proportional hazards regression analyses. The models were adjusted for a priori determined covariates based on established risk factors for VA and used in three separate models: Model 1 was adjusted for age, sex, and BMI, and Model 2 was additionally adjusted for CAD, HF, eGFR, and ICD indication. In Model 3, we additionally adjusted for QRS duration, QRS axis, presence of Q wave, and BBB in patients with native QRS on ECG. Sensitivity, specificity, and likelihood ratios were calculated for the ability of fQRS to predict VA. Kaplan–Meier plots were used to visualize the proportion of patients with events over time. Stata software (version 16, Statacorp.) was used to perform all analyses. For all statistical tests, p-value of less than 0.05 was considered significant.

TABLE 1 Baseline characteristics of the study population

Age	66.1 ± 12.0
Sex (male)	378 (82.5%)
Body mass index, kg/m^2	27.8 ± 4.7
Systolic blood pressure, mm Hg	126 ± 21
Diabetes mellitus	91 (19.8%)
Estimated glomerular filtration rate, ml/min/1.73 m^2	74 ± 23.4
Coronary artery disease	291 (64.4%)
History of acute myocardial infarction	260 (57.0%)
History of heart failure	370 (80.8%)
Left ventricular ejection fraction, %	40 ± 13
New York Heart Association class III-IV	49 (10.7%)
Cardiomyopathy	30 (6.6%)
Previous ventricular arrhythmias[a]	257 (56.2%)
History of atrial fibrillation	188 (41.2%)
ICD indication (secondary)	223 (48.6%)
Baseline ECG Parameters[b]	
Bundle branch block	80 (23.8%)
QRS duration (ms)	106 [94, 126]
QRS axis (degrees)	7 [−24, 38]
QTc duration (ms)	433 ± 34

Note: Data is shown as n (%), mean ± SD or median [Q1, Q3].
[a]Including episodes registered before ICD implantation.
[b]In patients with native QRS on baseline ECG (n = 336).

3 | RESULTS

3.1 | Baseline characteristics

Of 495 patients enrolled in the SMASH 1 Study, 36 (7%) patients were excluded from the current analysis due to missing baseline ECG, low-quality ECG recordings, and the presence of iRBBB (Figure 1). The 459 patients included in this analysis were aged 66 ± 12 years, 8% were male, and BMI was 28 ± 5 kg/m^2 (Table 1). Moreover, 20% had DM and 64% had established CAD, including 57% of the total population having a prior myocardial infarction (MI). In total, 12 patients had underlying arrhythmogenic right ventricular cardiomyopathy, 12 patients had hypertrophic cardiomyopathy, and 6 patients had channelopathy. Mean LVEF was 40% ± 13% in the total study cohort with LVEF 38% ± 13% in patients with primary ICD indication. In total, 81% of the population reported a diagnosis of HF and 11% were classified in NYHA functional class 3 or 4. The indication for ICD was primary prevention in 51% of

the population, 27% had CRT-D, and the time from implantation to study inclusion in the SMASH 1 Study was 5.0±6.7 years.

3.2 | Predictors of fQRS

In the baseline ECG, 217 (47%) patients had narrow QRS, 119 (26%) had wide QRS, and 123 (27%) had paced QRS, among whom 100 (81%) had biventricular pacing. fQRS was present in 52 patients (11%), and of these 35 (67%) had narrow QRS, 10 (19%) had wide QRS, and 7 (13%) had paced QRS. The adjudicators agreed on the ECG interpretation of fQRS in 99% of the cases. Patients with fQRS were older (69±11 vs 66±12 years, $p = .04$), had higher prevalence of CAD (86% vs 62%, $p < .001$), and were more likely to have established CAD (86% vs 62%, $p < .001$) than patients without fQRS (Table 2). Patients with fQRS had more frequently ICD implantation for secondary prevention (65% vs. 46%, $p = .01$) and more frequently experienced an episode of VT or VF prior to study enrollment (83% vs. 53%, $p < .001$). Other baseline characteristics, like gender, prevalence of HF, and LVEF, were comparable between patients with fQRS and without fQRS. Among patients with native QRS ($n = 336$), there were no significant differences in measurements of established ECG parameters between patients with versus without fQRS, including QRS duration, QRS axis, QTc, prevalence of BBB, and heart rate.

In a multivariable prediction model with all patients, history of CAD and a secondary prevention ICD indication remained independently associated with the presence of fQRS (Table S1).

3.3 | fQRS in association with incident ventricular arrhythmias and appropriate ICD therapy

During a mean follow-up of 3.1±0.7 years, 126 patients (28%) had at least one registered episode of VA, including 115 patients with sustained VT, 40 with VF, and 110 with appropriate ICD therapy. The presence of fQRS in the baseline ECG was associated with a higher risk of time to the first event of incident VA or appropriate ICD therapy: HR 3.41 (95% CI 2.27–5.13), $p < .001$ (Table 3, Figure 3). fQRS remained associated with the primary endpoint after adjusting for age, sex, and BMI (Model 1; HR 3.28 [95% CI 2.18–4.94], $p < .001$) and after additionally adjusting for CAD, HF, eGFR, and ICD indication (Model 2; HR 2.60 [95% CI 1.69–4.01], $p < .001$). fQRS also remained associated with the primary endpoint after additionally adjusting for established ECG parameters in patients with native QRS, including QRS duration, QRS axis, presence of Q wave, and BBB on baseline ECG (Model 3; HR 2.79 [95% CI 1.71–4.54], $p < .001$). The association between fQRS and the primary endpoint was consistent between the two study sites (HR 3.37 [95% CI 1.98–5.73] and HR 3.63 [95% CI 1.89–6.98],

TABLE 2 Baseline characteristics of patients with and without fQRS

	fQRS not present $n = 407$	fQRS present $n = 52$	p-value
Age	65.7±12.1	69.3±11.4	.04
Sex (female)	73 (17.9%)	7 (13.7%)	.46
Body mass index, kg/m^2	27.8±4.7	27.9±4.9	.82
Systolic blood pressure, mmHg	125±20	127±22	.50
Diabetes mellitus	80 (19.7%)	11 (21.2%)	.80
Estimated glomerular filtration rate, ml/min/1.73m^2	74±23	71±25	.42
Coronary artery disease	247 (61.6%)	44 (86.3%)	<.001
History of acute myocardial infarction	218 (54.0%)	42 (80.8%)	<.001
History of heart failure	325 (80.0%)	45 (86.5%)	.26
History of atrial fibrillation	162 (40.1%)	26 (50.0%)	.17
Left ventricular ejection fraction, %	41±13	38±12	.21
New York Heart Association class III-IV	44 (10.8%)	5 (9.6%)	.79
Cardiomyopathy	30 (7.4%)	0 (0.0%)	.04
Previous ventricular arrhythmias[a]	214 (52.8%)	43 (82.7%)	<.001
ICD indication (secondary)	189 (46.4%)	34 (65.4%)	.01
Baseline ECG Parameters[b]			
Bundle branch block	72 (24.7%)	8 (17.8%)	.31
QRS duration (ms)	106 [94, 130]	112 [100, 118]	.70
QRS axis (degrees)	7 [−24, 38]	12.0 [−21, 44]	.59
QTc duration (ms)	432±35	433±28	.91

Note: Data is shown as n (%) or mean±SD.

[a]Including episodes registered before ICD implantation.

[b]In patients with native QRS on baseline ECG ($n = 336$).

TABLE 3 Cox regression analyses for the association between fQRS and incident ventricular arrhythmia (N = 126); univariate and multivariate models

	Unadjusted model			Model 1[a] (*n* = 450)			Model 2[b] (*n* = 439)			Model 3[c] (patients with native QRS; *n* = 293)		
	Hazard ratio [95% CI]	*Z*-value	*p*-value	Hazard ratio [95% CI]	*Z*-value	*p*-value	Hazard ratio [95% CI]	*Z*-value	*p*-value	Hazard ratio [95% CI]	*Z*-value	*p*-value
QRS fragmentation	3.41 [2.27–5.13]	5.9	<.001	3.28 [2.18–4.94]	5.7	<.001	2.60 [1.69–4.01]	4.3	<.001	2.79 [1.71–4.54]	4.1	<.001
Age				1.18 [1.00–1.40]	2.0	.046	1.08 [0.88–1.31]	0.7	.46	1.23 [0.97–1.56]	1.7	.08
Sex				1.08 [0.68–1.73]	0.3	.75	1.37 [0.85–2.20]	1.3	.19	1.05 [0.61–1.82]	0.2	.85
Body mass index, kg/m^2				1.02 [0.69–1.50]	0.1	.94	1.01 [0.69–1.50]	0.1	.94	0.95 [0.60–1.51]	−0.2	.82
Coronary artery disease							1.55 [0.97–2.47]	1.8	.07	1.32 [0.72–2.41]	0.9	.37
History of heart failure							1.46 [1.17–1.81]	3.4	.001	1.66 [1.29–2.12]	4.0	<.001
eGFR, ml/min/1.73 m^2							1.04 [0.96–1.13]	1.0	.34	1.10 [0.99–1.21]	1.8	.08
ICD indication (secondary)							1.41 [0.96–2.06]	1.8	.08	1.63 [1.02–2.61]	2.0	.04
QRS duration										0.90 [0.79–1.02]	−1.7	.09
QRS axis										0.99 [0.95–1.02]	−0.7	.47
Q wave presence										1.08 [0.67–1.72]	0.3	.76
Bundle branch block										1.41 [0.70–2.84]	1.0	.33

[a]Model 1: adjusted for age, sex, and body mass index.

[b]Model 2: adjusted for Model 1 + coronary artery disease, heart failure, estimated glomerular filtration rate, and ICD indication.

[c]Model 3: adjusted for Model 2 + QRS duration, QRS axis, presence of Q wave, and bundle branch block in patients with native QRS on baseline ECG.

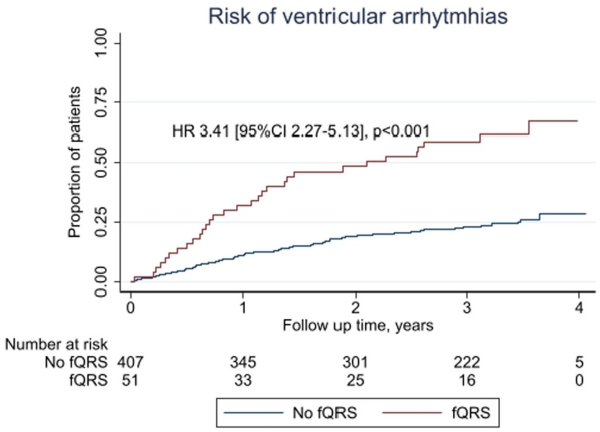

FIGURE 3 Survival analysis of time to ventricular arrhythmia and appropriate ICD therapy in patients with and without QRS fragmentation present in the baseline ECG

p-for-interaction = .93; Table S2). The association between fQRS and the primary endpoint was independent of the presence of BBB on baseline ECG (among patients with native QRS, *p*-for-interaction = .76). The presence of fQRS had specificity 94% and sensitivity 25% with a positive likelihood ratio of 4.2 for incident VA or appropriate ICD therapy.

In a sensitivity analysis including all-cause death ($N = 68$) in a combined outcome with incident VA or appropriate ICD therapy, fQRS was associated with the risk of incident VA or death: HR 2.65 [95% CI 1.82–3.87], $p < .001$, and this persisted in the adjusted models.

3.4 | fQRS and outcome according to ICD indication

fQRS was associated with the primary endpoint irrespective of ICD indication, but there was a stronger association among patients with a primary prevention ICD indication than patients with a secondary prevention ICD indication (HR 6.05 [95% CI 3.16–11.60] versus HR 2.39, [95% CI 1.41–4.04], respectively, *p*-for-interaction = .047). Among patients with a primary ICD indication, the presence of fQRS was associated with established CAD ($p = .048$) andprior MI ($p = .02$). fQRS and pathological Q waves were the only variables associated with the primary endpoint in multivariable models in these patients (Table S3). The association between fQRS and the primary endpoint in primary prevention was not modified by the presence or absence of CAD (p-for-interaction 0.39): HR 4.78 [95% CI 2.33–9.84], $p < .001$ and HR 8.63 [95% CI 1.85–40.3], $p = .006$, respectively.

3.5 | fQRS in association with ventricular tachycardia and ventricular fibrillation

When assessing the association between fQRS and components of the primary endpoint, we found an association between fQRS and incident VT (HR 3.04 [95% CI 1.96–4.71], $p < .001$), which persisted in the adjusted Model 3 ($p = .003$). fQRS was also associated with incident VF (HR 2.56, [95% CI 1.18–5.57], $p = .02$), which persisted after adjustments ($p = .03$). Among patients with incident VT or VF, 88% were appropriately treated with ATP or DC shock, and fQRS also predicted appropriate ICD therapy: HR 3.56, [95% CI 2.31–5.48], $p < .001$), which persisted after adjustments ($p < .001$).

In patients with primary ICD indication, fQRS was strongly associated with incident VT (HR 4.79 [95% CI 2.30–9.98], $p < .001$) and VF (HR 6.85 [95% CI 2.18–21.53], $p = .001$), and these associations persisted in the adjusted Model 3 ($p < .001$ for VT and $p = .004$ for VF).

4 | DISCUSSION

In this study, we found a strong and independent association between the presence of fQRS and incident VA and appropriate ICD therapy. Patients with fQRS had a threefold increased risk for developing VA irrespective of established risk factors and ECG parameters. These results suggest that interpreting ECGs for fragmentation of the QRS complex may improve risk stratification for SCD.

4.1 | Pathophysiology reflected by QRS fragmentation

In our study of unselected patients treated with ICD, fQRS was present in 11%, which is lower than in previous populations by Das: 23% in patients with ischemic and nonischemic cardiomyopathy, excluding patients with paced rhythm and inherited channelopathies (Das et al., 2010) and 35% in patients with CAD (Das et al., 2006). As these populations were selected for by the etiology of the cardiomyopathy or by the presence of CAD, this is not surprising and highlights the additional value provided by our study of non-selected patients with ICD. The presence of fQRS in our study was associated with established CAD, prior MI and a primary indication for ICD. There were limited associations to demographics and other comorbidities. The underlying mechanism causing the QRS complex to fragment is not completely understood. Early studies have focused on fQRS as a marker of myocardial scar. In agreement with our findings, Das et al. reported that fQRS represents myocardial scar, as indicated by regional perfusion abnormalities detected by nuclear stress test (Das et al., 2006). fQRS was superior to Q waves in detecting myocardial scars with significantly higher sensitivity and negative predictive value: 86% and 93%, respectively, for fQRS and 36% and 71%, respectively, for Q waves. Ratheendran et al. also reported an association between fQRS and myocardial scar in patients with hypertrophic cardiomyopathy (HCM; Ratheendran et al., 2020). They reported a higher incidence of delayed gadolinium enhancement on cardiac magnetic resonance imaging (CMR), indicating the presence of myocardial scar, in patients with fQRS compared with patients without fQRS (85% vs. 10%, respectively). The presence of fQRS had an 85% sensitivity and 90% specificity in detecting myocardial scar on CMR.

The criteria for fQRS are different for patients with narrow and wide QRS. Both increased QRS duration and presence of fQRS represent a conduction delay and a depolarization abnormality in the left ventricle. Interestingly, we found no association between presence of fQRS and QRS duration or axis among patients with native QRS, suggesting that this phenomenon is independent of other electrophysiological measures of ventricular depolarization. Hence, the exact pathophysiology behind fQRS remains unknown and is an area for future studies to investigate.

4.2 | QRS fragmentation as a predictor for ventricular arrhythmias

There is an unmet need to identify patients at risk of SCD. The current patient selection for treatment with ICD has major limitations and suffers from both poor sensitivity and specificity. Novel approaches to identify patients at risk are typically complicated and involve advanced imaging and deep phenotyping (de Haan et al., 2011; Lee Daniel & Goldberger, 2013). In this study, we found fQRS, which is an easily available parameter from standard 12-lead ECG, to be associated with a threefold increased risk of VA, with a specificity of 94%. In agreement with previous studies (Igarashi et al., 2017; Kucharz & Kułakowski, 2020; Ozcan et al., 2014), fQRS was superior to, and independent of, established clinical risk factors and ECG parameters with respect to VA. Our results, however, demonstrate a stronger association between fQRS and VA, which may relate to our sample size being larger and more heterogeneous. Furthermore, our outcome measure was specific for ventricular arrhythmias, as opposed to broader composite endpoints used in other studies (Engstrom et al., 2022).

In our study, fQRS was significantly associated with both incident VT and VF separately, although the number of events was lower and confidence intervals wider for VF. Importantly, the association between fQRS and VA was independent of QRS duration and the presence of BBB, which may suggest that the arrhythmogenicity reflected by fQRS is a result of heterogeneous ventricular depolarization rather than a conduction delay per se.

The incidence of sudden death has declined in heart failure with reduced ejection fraction (HFrEF) as a result from the cumulative benefit of evidence-based treatment (Shen et al., 2017). The effect of ICD treatment as primary prevention in patients with nonischemic HFrEF was investigated in the Danish trial (Køber et al., 2016). In this trial, ICD therapy was not superior to usual clinical care with respect to long-term rate of all-cause death, although there was a reduction in SCD. This demonstrates the importance of developing better risk stratification tools in patients considered for primary prevention ICD. A previous study of patients with primary prevention ICD indication did not find an association between fQRS and risk of either all-cause mortality or arrhythmic mortality (Cheema et al., 2010). However, this study did not investigate the association with incident VT or VF. In our study, fQRS was strongly predictive of VA in patients with a primary prevention indication for ICD, and this association was stronger than among secondary prevention patients. Moreover, the association between fQRS and VA in primary prevention was irrespective of the presence of CAD, suggesting that in nonischemic HFrEF, where the effect of ICD therapy is more uncertain, there may be a role for fQRS in identifying patients with increased risk of cardiac arrest (Maheshwari et al., 2013).

4.3 | Strengths

This was a large, investigator-initiated multicenter study with long follow-up. The main finding of the study was consistent across the study sites. Reporting SCD and whether it is caused by malignant VA can be challenging and inaccurate. Several studies have defined SCD according to guidelines as a sudden, unexpected, and nontraumatic death of healthy individuals occurring within one hour after onset of symptoms (Priori et al., 2015; Zipes et al., 2006). If it is unwitnessed, it is still defined as SCD if the individual was healthy 24 h before the death occurred (Priori et al., 2015). In our study, we have more accurate definitions of VA beyond clinical observations, as our arhythmical events are actual events documented and interrogated by experienced cardiac electrophysiologists. As ICD treatment prevents SCD caused by VA (Bardy et al., 2005; Buxton et al., 1999), we believe the association between fQRS and incident VA in our study is likely to translate to an association between fQRS and SCD. This is supported by the fact that 88% of the patients with VA also were treated with ATP or DC shock as programmed in the ICD algorithms.

4.4 | Limitations

We included patients treated with ICD, who are a high-risk population for VA. Our results can, therefore, not directly be applied to other patient populations or the general population who are at much lower risk. We had an uneven distribution of sex across our cohort with 83% males, although this typically represents the population with ICD. ECGs were obtained upon study enrollment, which was mean 5.0 ± 6.7 years after ICD implantation. Our patients were included at two centers, and all ECGs were analyzed by two independent adjudicators with the risk of observer bias; however, there was 99% agreement in the adjudication of fQRS. ECG recordings were done at paper speed 50 mm/s, as opposed to standard 25 mm/s, due to local routines. We excluded 19 (4%) patients because of missing, or non-interpretable ECG due to artifacts. However, this exclusion was random and not likely to influence our results. ICD programming was not standardized and may thus infer some variations in the sensitivity of VT detections. However, physicians were blinded to the fQRS status and any variations are not likely to bias the results. Patients who died from an unknown cause and who did not have their ICD interrogated post-mortem could potentially have died from VA. However, we demonstrate similar results when adding all-cause death to the primary outcome measure.

5 | CONCLUSIONS

fQRS is a highly feasible and easily available tool that can aid in assessing the risk for subsequent VA. Our study demonstrates that the presence of fQRS in ECG is associated with a threefold higher risk of developing VA, beyond established risk factors. The association appeared strongest in patients with a primary prevention ICD indication where there is an unmet need for novel risk assessment tools. Evaluating fQRS may prove to become such a tool. Automated detection of fQRS as a part of the computer algorithm interpretation of ECG, to reduce the risk of misinterpreting normal variant such as iRBBB, is a potential way forward. This could aid clinicians in detecting fQRS easily and potentially support decision-making regarding ICD implantation in high-risk patients.

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CONFLICT OF INTEREST

Dr. Røsjø has received personal fees from Thermo Fischer BRAHMS, CardiNor and SpinChip Diagnostics. Dr Kjekshus has served on advisory boards and received speaker fees from Bayer and Pfizer. Dr. Omland has served on advisory boards for Abbott Diagnostics, Roche Diagnostics, and Bayer and has received research support from Abbott Diagnostics, Novartis, Roche Diagnostics, Singulex, and SomaLogic via Akershus University Hospital, and speaker's or consulting honoraria from Roche Diagnostics, Siemens Healthineers, and CardiNor. Dr. Myhre has served on advisory boards and received speaker fees from AmGen, AstraZeneca, Bayer, Boehringer Ingelheim, Novartis, and Novo Nordisk. All other authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ETHICAL APPROVAL

The study was conducted in accordance with the Declaration of Helsinki. All patients provided informedwritten consent, and the study was approved by the Regional Ethics Committee (2015/2080) and the local DataProtection Officers at the institutions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplementary Material

to

QRS fragmentation is associated with increased risk of ventricular arrhythmias in high-risk patients; data from the SMASH 1 Study

Supplemental Table 1. Predictors of fQRS in baseline ECG assessed by multivariable logistic regression analysis

	Total population			Patients with native QRS in baseline ECG		
	Hazard Ratio [95% CI]	**Z-value**	**P-value**	**Hazard Ratio [95% CI]**	**Z-value**	**P-value**
Age	1.17 [0.84-1.62]	0.9	0.35	1.15 [0.80-1.66]	0.8	0.44
Gender	0.98 [0.41-2.37]	-0.04	0.97	1.07 [0.42-2.72]	0.2	0.88
Body mass index, kg/m^2	1.20 [0.61-2.36]	0.5	0.61	1.25 [0.59-2.64]	0.6	0.56
Coronary artery disease	3.31 [1.37-7.98]	2.7	0.01	2.39 [0.86-6.64]	1.7	0.09
History of heart failure	0.99 [0.73-1.33]	-0.1	0.92	1.07 [0.76-1.49]	0.4	0.70
Estimated glomerular filtration rate, $ml/min/1.73m^2$	0.99 [0.85-1.15]	-0.2	0.88	1.02 [0.85-1.23]	0.2	0.81
ICD indication	2.15 [1.13-4.12]	2.3	0.02	1.90 [0.89-4.06]	1.7	0.10
QRS duration				1.11 [0.91-1.36]	1.1	0.29
QRS axis				1.02 [0.95-1.10]	0.7	0.50
Q wave presence				1.32 [0.62-2.83]	0.7	0.47
Bundle branch block				0.53 [0.17-1.65]	-1.1	0.27

Supplemental Table 2. Cox regression model for the two study centers, univariate and multivariate model.

	Unadjusted model	Adjusted model*
	Hazard Ratio, [95% Conf. Interval]	**Hazard Ratio, [95% Conf. Interval]**
Akershus University Hospital	3.37, [1.98-5.73]	2.39, [1.21-4.72]
Stavanger University Hospital	3.63, [1.89-6.98]	3.79, [1.75-8.20]
P-for interaction	*P=0.93*	

**Adjusted for age, gender, body mass index, coronary artery disease, heart failure, e*stimated glomerular filtration rate, *ICD indication (primary vs. secondary), QRS duration, QRS axis, presence of Q-wave, and bundle branch block on baseline ECG in patients with native QRS*

Suppl. Table 3 Cox regression analyses for the association between fQRS and incident ventricular arrythmia in patients with primary ICD indication; univariate and multivariate models.

	Unadjusted model			Model 1 *			Model 2 **			Model 3 *** *(Patients with native QRS only)*		
	Hazard Ratio [95% CI]	**Z-value**	**P-value**	**Hazard Ratio [95% CI]**	**Z-value**	**P-value**	**Hazard Ratio [95% CI]**	**Z-value**	**P-value**	**Hazard Ratio [95% CI]**	**Z-value**	**P-value**
QRS fragmentation	6.05 [3.16-11.60]	5.4	<0.001	5.88 [3.06-11.29]	5.3	<0.001	5.47 [2.83-10.56]	5.1	<0.001	11.22 [4.20-29.99]	4.8	<0.001
Age				1.11 [0.86-1.44]	0.8	0.42	1.07 [0.79-1.44]	0.4	0.66	1.09 [0.70-1.68]	0.4	0.70
Sex				0.39 [0.14-1.08]	-1.8	0.07	0.56 [0.19-1.61]	-1.1	0.28	0.40 [0.09-1.79]	-1.2	0.23
Body mass index, kg/m^2				1.10 [0.66-1.84]	0.4	0.72	1.05 [0.63-1.75]	0.2	0.86	1.06 [0.52-2.18]	0.2	0.86
Coronary artery disease							1.83 [0.91-3.68]	1.7	0.09	2.07 [0.68-6.26]	1.3	0.20
History of heart failure							1.36 [0.91-2.02]	1.5	0.13	1.48 [0.91-2.41]	1.6	0.12
eGFR, ml/min/1.73m^2							1.10 [0.97-1.24]	1.5	0.14	1.14 [0.96-1.37]	1.5	0.14
QRS duration										0.94 [0.74-1.19]	-0.5	0.59
QRS axis										0.97 [0.93-1.01]	-1.3	0.18
Q wave presence										2.83 [1.19-6.71]	2.4	0.02
Bundle branch block										0.86 [0.22-3.38]	-0.2	0.83

** Model 1: adjusted for age, sex and body mass index*

*** Model 2: adjusted for Model 1 + coronary artery disease, heart failure* and estimated glomerular filtration rate

****Model 3: adjusted for Model 2 + QRS duration, QRS axis, presence of Q-wave, and bundle branch block on baseline ECG in patients with native rhythm on ECG*

II

Paper II

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CLINICAL TRIAL

CLINICAL CARDIOLOGY WILEY

N-terminal pro-B-type natriuretic peptide for prediction of ventricular arrhythmias: Data from the SMASH study

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Abstract

Background: Elevated N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations predict heart failure (HF) and mortality, but whether NT-proBNP predicts ventricular arrhythmias (VA) is not clear.

Hypothesis: We hypothesize that high NT-proBNP concentrations associate with the risk of incident VA, defined as adjudicated ventricular fibrillation or sustained ventricular tachycardia.

Methods: In a prospective, observational study of patients treated with implantable cardioverter defibrillator (ICD), we analyzed NT-proBNP concentrations at baseline and after mean 1.4 years in association to incident VA.

Results: We included 490 patients (age 66 ± 12 years, 83% men) out of whom 51% had a primary prevention ICD indication. The median NT-proBNP concentration was 567 (25–75 percentile 203–1480) ng/L and patients with higher concentrations were older with more HF and ICD for primary prevention. During mean 3.1 ± 0.7 years, 137 patients (28%) had ≥1 VA. Baseline NT-proBNP concentrations were associated with the risk of incident VA (hazard ratio [HR]: 1.39, 95% confidence interval [95% CI]: 1.22–1.58, $p < .001$), HF hospitalizations (HR: 3.11, 95% CI: 2.53–3.82, $p < .001$), and all-cause mortality (HR: 2.49, 95% CI: 2.04–3.03, $p < .001$), which persisted after adjusting for age, sex, body mass index, coronary artery disease, HF, renal function, and left ventricular ejection fraction. The association with VA was stronger in secondary versus primary prevention ICD indication: HR: 1.59 (95% CI: 1.34–1.88 C-statistics 0.71) versus HR: 1.24, 95% CI: 1.02–1.51, C-statistics 0.55), p-for-interaction = 0.06. Changes in NT-proBNP during the first 1.4 years did not associate with subsequent VA.

Conclusions: NT-proBNP concentrations are associated with the risk of incident VA after adjustment for established risk factors, with the strongest association in patients with a secondary prevention ICD indication.

KEYWORDS

ICD, implantable cardioverter defibrillator, N-terminal pro-B-type natriuretic peptide, NT-proBNP, sudden cardiac death, ventricular arrhythmias

1 | INTRODUCTION

Ventricular arrhythmia (VA) is an important cause of sudden cardiac death (SCD) globally.[1,2] Prediction of risk for VA and patients selection for treatment with implantable cardioverter defibrillator (ICD) are challenging due to a large number of heart disease conditions that can result in VA and subsequently SCD.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a natriuretic peptide secreted by ventricular cardiomyocytes in response to cardiac stress mainly due to congestive heart failure (HF).[3] Elevated levels of NT-proBNP are predictive of poor prognosis in patients with HF, asymptomatic left ventricular (LV) dysfunction and coronary artery disease (CAD).[4,5] Previous studies have demonstrated that higher NT-proBNP concentrations are associated with increased risk of SCD in patients with chronic HF, ischemic heart disease, hypertrophic cardiomyopathy, and in the general population.[6–9] Most studies have analyzed NT-proBNP in association with clinically suspected SCD[6,8,10] and few have investigated the association with recordings of VA,[7,11] which is an outcome measure more relevant for risk stratification and patients selection for ICD treatment. HF with reduced LV ejection fraction (LVEF) is the most frequent primary prevention ICD indication, and these patients therefore typically have higher NT-proBNP concentrations than patients with a secondary prevention ICD indication.

The primary aim of this study was to assess the association between NT-proBNP and device-recorded and adjudicated incident VA. The secondary aim was to assess the association between NT-proBNP and the risk of HF hospitalization and death.

2 | METHODS

2.1 | Study design and study population

SMASH (Scandinavian Multicenter study to Advance risk Stratification in Heart disease – ventricular arrhythmia) 1 is a prospective, observational, multicenter study.[12] Patients treated with ICD who were ≥18 years old with life expectancy >2 years were screened for inclusion (inclusion criteria are summarized in Supporting Information: Figure 1). Study participants were included during regular outpatient visits at the Departments of Cardiology at Akershus University Hospital and Stavanger University Hospital between August 2016 and March 2018. All patients were invited to a follow-up visit between 1 and 2 years after inclusion.

Patients underwent a physical examination at the baseline and follow-up visit including measurement of blood pressure (average of the second and third measurements) and heart rate after 5 min rest. Body weight and height were measured, and body mass index (BMI) was calculated. Information regarding previous medical history and New York Heart Association (NYHA) functional class was obtained from a structured interview and by a thorough review of the electronic health records. Glomerular filtration rate (eGFR) was estimated from creatinine measured in routine blood samples. The most recent measurement of LVEF by echocardiography or cardiac magnetic imaging was recorded. CAD was defined as established chronic coronary syndrome or previously experienced acute coronary syndromes.

2.2 | Analysis of NT-proBNP

At both visits, patients donated blood specimens by venipuncture, performed by trained study nurses. Samples for the study biobank were temporarily stored at 4°C, centrifuged at 2000*g* for 10 min and then transferred into aliquots that were frozen and stored at −80°C at Akershus University Hospital. Serum samples that had not previously been thawed were used to measure NT-proBNP, which was analyzed by the electrochemiluminescence immunoassay Elecsys on the Cobas e 801 platform (Roche Diagnostics). The coefficients of variations reported by the manufacturer were 2.5% at 127 ng/L and 1.3% at 1706 ng/L.

2.3 | Outcome measures

The primary outcome in the SMASH study was incident VA, defined as episodes of ventricular tachycardia (VT) or ventricular fibrillation (VF) resulting in appropriately delivered ICD therapies, that is, electrical shock or antitachycardia pacing, or sustained ventricular tachyarrhythmia (>100 b.p.m. and >30 s). Events with VA were obtained from ICD recordings and adjudicated by experienced cardiac electrophysiologists that were blinded to study biomarker concentrations. Study investigators also reviewed the ICD recordings and the reports in the electronic healthcare record and validated real events from artifacts and ensured that appropriate therapies were separated from inappropriate ICD therapies. Only events validated as real VAs were included as outcomes in the study. HF hospitalization and death from any cause were secondary endpoints, registered by review of the electronic healthcare records of the patients, with linkage to the National Death Registry.

2.4 | Statistical analysis

Values are reported as *N* (%) and median (Quartile 1 to Quartile 3) for skewed and mean ± SD for normally distributed variables. NT-proBNP had a non-normal distribution according to the Shapiro–Wilk normality test, and log-transformed values were therefore used in all regression analyses. Categorical and continuous variables were compared using the χ^2 test for binary variables, analysis of variance for parametric continuous variables, and the Kruskal–Wallis test for nonparametric continuous variables. Baseline characteristics were compared for trend across quartiles of baseline NT-proBNP using linear and logistic regression models. Independent predictors of higher baseline NT-proBNP concentrations were determined using multivariable linear regression analysis. The

associations between baseline concentrations of NT-proBNP and time to first event for each of the endpoints (incident VA, HF hospitalization, and death in separate analyses) were examined in unadjusted and adjusted Cox proportional hazard regression models. Multivariable Model 1 was adjusted for age, sex, and BMI, and Model 2 was additionally adjusted for CAD, HF, eGFR, and LVEF. Harrell's C-statistics was calculated to assess the performance of NT-proBNP to discriminate between patients based on time to event. We performed interaction analysis to determine whether the association between NT-proBNP and VA was different in patients with primary versus secondary prevention ICD indication. We used Kaplan–Meier plots to visualize the proportion of patients with endpoint events over time by quartiles of baseline NT-proBNP.

In patients with available NT-proBNP concentrations at the follow-up visit, we used Wilcoxon signed-rank test to analyze changes from the baseline samples. Relative changes in NT-proBNP from baseline to follow-up was calculated by dividing the follow-up concentration with the baseline concentration. This ratio was log-transformed and analyzed in landmark Cox regression models for events after the date of the follow-up. All statistical analyses were performed using Stata Software (version 17, Stata Corp.). A two-sided $p < .05$ was considered statistically significant.

3 | RESULTS

3.1 | Baseline characteristics

In the SMASH 1 Study, we included 495 patients treated with ICD, one withdrew from the study and among the remaining patients 490 (99%) had available study blood samples and were included in this analysis (Supporting Information: Figure 1). The mean age was 66 ± 12 years and 83% were men with a mean BMI of 28 ± 5 kg/m^2 and LVEF of 40 ± 13%. Most patients had comorbid conditions, including CAD (64%), previous acute myocardial infarction (AMI, 57%) and HF (80%). The time from ICD implantation to study inclusion was 5.1 ± 6.6 years. Two-hundred and fifty (51%) patients had a primary prevention ICD indication and 135 (28%) patients had cardiac resynchronization therapy with ICD indication. Baseline medications included 458 (94%) on β-blockers, 395 (81%) on renin–angiotensin system inhibitors, and 84 (17%) on antiarrhythmic drugs. Among patients with ICD for primary prevention, HF was the indication in 225 patients (90%; mean LVEF 35 ± 11%), whereas 25 patients (10%; mean LVEF 57 ± 6%) had a non-HF indication, predominantly cardiomyopathy ($n = 17$) (Supporting Information: Table 1).

3.2 | Predictors of higher NT-proBNP concentrations

The median (Q1–Q3) concentration of NT-proBNP in the total population was 567 (203–1480) ng/L. Patients with higher NT-proBNP concentrations were older, had lower BMI, lower LVEF, and higher NYHA functional class (Table 1). Patients with high NT-proBNP concentrations were also more likely to have a greater burden of comorbidities, including HF, diabetes, CAD, previous AMI, and worse renal function. In multivariable regression models, older age, lower BMI, history of HF, absence of cardiomyopathy, NYHA class III–IV, lower LVEF, and lower eGFR levels were independent predictors of higher NT-proBNP concentrations (Supporting Information: Table 2).

3.3 | NT-proBNP in association with incident VAs

During a mean follow-up of 3.10 ± 0.74 years, 137 (28%) patients experienced at least one episode of VA, among whom 126 had VT, 47 had VF, and 120 had appropriate ICD therapy. Higher NT-proBNP concentrations were associated with greater risk of time-to-first-event of incident VA: hazard ratio (HR): 1.39, 95% confidence interval (CI): 1.22–1.58 per log unit increase, $p < .001$ (Table 2). This association persisted after adjusting for age, sex, and BMI (HR: 1.37 [95% CI: 1.20–1.58], $p < .001$), and after additionally adjusting for CAD, HF, eGFR, and LVEF (HR: 1.22 [95% CI: 1.03–1.45], $p = .02$). Patients in the highest quartile of NT-proBNP had almost fourfold higher risk of VA compared with the lowest quartile (HR: 3.86 [95% CI: 2.10–7.10], $p < .001$) (Figure 1). The C-statistics for NT-proBNP in predicting VA was 0.62 [95% CI: 0.57–0.67].

3.4 | NT-proBNP and risk of VA in primary and secondary prevention ICD indication

Patients with a primary prevention ICD indication had higher NT-proBNP concentrations than patients with secondary prevention indication: median 761 (235–1818) ng/L versus 442 (192–1058) ng/L, $p < .001$ (Supporting Information: Table 1). There was a trend for a stronger association between NT-proBNP concentrations and incident VA in patients with a secondary prevention ICD indication (HR 1.59 [95% CI 1.34–1.88], $p < .001$) compared with patients with a primary prevention ICD indication (HR 1.24 [1.02–1.51], $p = .03$), $p = .06$ (Table 3 and Figure 1). In patients with a secondary prevention indication, the association between NT-proBNP and VA persisted in the fully adjusted model (HR 1.32 [1.02–1.70], $p = .04$), whereas it was attenuated and nonsignificant in primary prevention patients (HR 1.10 [0.87–1.40, $p = .46$). The C-statistics for patients with secondary prevention was 0.71 (95% CI: 0.64–0.77) and primary prevention 0.55 (95% CI: 0.47–0.63).

3.5 | NT-proBNP and associations with death and HF hospitalization

During follow-up, 87 patients (18%) experienced at least 1 hospitalization for HF and 76 (16%) patients died during follow-up,

TABLE 1 Baseline characteristics of patients according to baseline NT-proBNP quartiles.

	NT-proBNP Q1 *n* = 123	NT-proBNP Q2 *n* = 122	NT-proBNP Q3 *n* = 123	NT-proBNP Q4 *n* = 122	*p* for trend
NT-proBNP range, ng/L	16–203	207–567	568–1480	1488–35 000	
Age, years	57.9 ± 12.6	67.3 ± 9.9	68.3 ± 9.8	71.0 ± 12.8	<.001
Male sex	98 (79.7%)	107 (87.7%)	96 (78.0%)	106 (87.6%)	.35
BMI, kg/m^2	28.3 ± 5.0	28.9 ± 4.1	27.9 ± 4.9	25.7 ± 4.2	<.001
Systolic blood pressure, mmHg	125 ± 17	128 ± 20	125 ± 21	122 ± 23	.11
Diabetes mellitus	12 (9.8%)	23 (18.9%)	25 (20.3%)	34 (27.9%)	<.001
CAD	53 (43.8%)	86 (70.5%)	82 (68.3%)	88 (73.3%)	<.001
Previous AMI	40 (32.8%)	81 (66.4%)	74 (60.7%)	81 (66.9%)	<.001
HF	63 (51.2%)	102 (83.6%)	112 (91.1%)	114 (95.1%)	<.001
LVEF, %	50 ± 11	42 ± 11	36 ± 11	33 ± 12	<.001
NYHA Class III–IV	2 (1.6%)	16 (13.1%)	14 (11.4%)	20 (16.4%)	<.001
Cardiomyopathy	16 (13.0%)	5 (4.1%)	5 (4.1%)	8 (6.6%)	.06
Previous documentation of VA	66 (54.1%)	85 (69.7%)	68 (55.7%)	63 (51.6%)	.30
Primary ICD indication	57 (46.3%)	48 (39.3%)	66 (53.7%)	77 (63.6%)	<.001
Estimated GFR, ml/min/1.73m^2	86 ± 21	78 ± 22	72 ± 23	58 ± 22	<.001
Baseline medications					
β-blockers	106 (86.2%)	116 (95.1%)	119 (96.7%)	117 (95.9%)	.002
Angiotensin-converting enzyme inhibitors	53 (43.1%)	61 (50.4%)	72 (58.5%)	60 (49.2%)	.19
Angiotensin II receptor blockers	28 (22.8%)	43 (35.2%)	34 (27.6%)	44 (36.1%)	.08
Mineralocorticoid receptor antagonists	21 (17.1%)	46 (37.7%)	59 (48.0%)	55 (45.1%)	<.001
Antiarrhythmic drugs	10 (8.1%)	18 (14.8%)	28 (22.8%)	28 (23.0%)	<.001

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index; CAD, coronary artery disease; GFR, glomerular filtration rate; HF, heart failure; ICD, implantable cardioverter defibrillator; LVEF, Left ventricular ejection fraction; NT-proBNO, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; VA, ventricular arrhythmia.

including 35 classified as cardiovascular death. Greater concentrations of NT-proBNP were associated with a higher risk of HF hospitalization (HR 3.11 [2.53–3.82], $p < .001$; C-statistics 0.85) (Table 2 and Figure 2A) and this persisted in adjusted models. NT-proBNP concentrations were associated with all-cause mortality (HR 2.49 [95% CI 2.04–3.03], $p < .001$; C-statistics 0.82) (Table 2 and Figure 2B), which persisted in adjusted models.

3.6 | Change in NT-proBNP measurements from baseline to follow-up

In total, 459 (94%) patients attended the follow-up visit. Among the 30 nonattending patients, 25 were dead. Blood samples were collected in 411 (84%) patients, mean 1.4 ± 0.5 years after the baseline visit. Baseline characteristics of patients with and without follow-up NT-proBNP measurements are presented in Supporting Information: Table 3. The median NT-proBNP concentration at the follow-up visit was 469 (171–1202) ng/L, which was not significantly different from the baseline concentrations ($p = .31$). The relative change in NT-proBNP from baseline to follow-up was median −2% (−35% to 36%). Patients with greater increases in NT-proBNP between the visits had higher baseline blood pressure, higher baseline LVEF and more frequently a secondary indication for ICD (Supporting Information: Table 4). Changes in NT-proBNP were not associated with subsequent incident VA ($N = 46$; HR: 1.00 [95% CI: 0.66–1.52] $p = .98$). Greater changes in NT-proBNP associated with an increased risk of subsequent hospitalization for HF ($N = 34$; HR: 1.73 [95% CI: 1.03–2.90], $p = .04$ and all-cause death ($N = 42$; HR: 1.71 [95% CI: 1.05–2.77], $p = .03$) in the fully adjusted model. These results were consistent when analyzing absolute changes in NT-proBNP.

4 | DISCUSSION

We report the following main findings: (1) Higher concentrations of NT-proBNP predict the risk of incident VA, with an almost fourfold increased risk in patients with NT-proBNP in the highest quartile (>~1500 ng/L) compared with the lowest quartile (<~200 ng/L). (2)

TABLE 2 Association between NT-proBNP and the risk of VA, hospitalization for HF, and all-cause mortality.

	C-statistics Harrell's C (95% CI)	Cox regression – Unadjusted model		Cox regression – Multivariable model 1[a]		Cox regression – Multivariable model 2[b]	
		HR (95% CI)	*p*	HR (95% CI)	*p*	HR (95% CI)	*p*
VA (*n* = 137)	0.62 (0.57–0.67)	1.39 (1.22–1.58)	<.001	1.37 (1.20–1.58)	<.001	1.22 (1.03–1.45)	.02
HF hospitalization (*n* = 87)	0.85 (0.81–0.89)	3.11 (2.53–3.82)	<.001	3.38 (2.69–4.24)	<.001	3.04 (2.33–3.97)	<.001
All-cause mortality (*n* = 76)	0.82 (0.77–0.87)	2.49 (2.04–3.03)	<.001	2.33 (1.86–2.92)	<.001	1.96 (1.50–2.58)	<.001

Note: Analyzed by proportional Cox regression per log unit increase of NT-proBNP in association to events in unadjusted model and after adjustments for risk factors in two separate models. Also presented is Harrell's C-statistics for the unadjusted model.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; GFR, glomerular filtration rate; HF, heart failure; HR, hazard ratio; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VA, ventricular arrhythmia.

[a]Adjusted for age, sex, and BMI.

[b]Adjusted for age, sex, BMI, CAD, HF, estimated GFR, and LVEF.

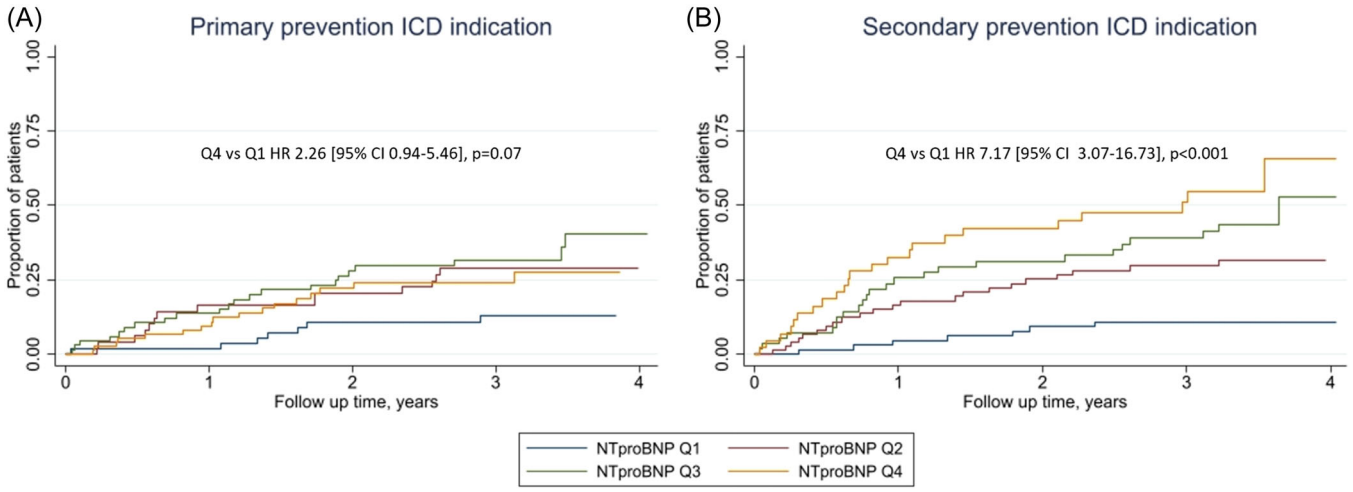

FIGURE 1 Association between baseline concentrations of N-terminal-pro B-type natriuretic peptide (NT-proBNP) and time to ventricular arrhythmia in patients with (A) primary prevention implantable cardioverter defibrillator (ICD) indication and (B) secondary prevention ICD indication. Stratified by quartiles of NT-proBNP and *p* is for Quartile 4 versus Quartile 1.

This association was independent of established risk factors for cardiac arrest such as age, sex, CAD, renal function, and most importantly, LVEF. (3) The association appeared to be stronger in patients with secondary prevention than primary prevention ICD indication. (4) There was no association between change in NT-proBNP levels over ~1.5 years and the risk of subsequent VA.

4.1 | NT-proBNP as a predictor for major cardiovascular events

Elevated levels of circulating NT-proBNP are common in patients with HF and measurements are recommended for diagnostic and prognostic purposes.[6,13–16] The association between NT-proBNP levels and cardiovascular risk has also been demonstrated in lower risk cohorts, including community-based studies of individuals free of HF.[8,17,18] In our study we extend these findings to patients treated with ICD at very high cardiovascular risk by showing a strong association between higher baseline NT-proBNP concentrations and an increased risk of VA, HF-hospitalization, and all-cause death. NT-proBNP performed better at predicting the risk of HF hospitalization and mortality compared with VA risk. This finding is in line with previous studies suggesting NT-proBNP to be a strong prognostic marker of worsening HF status and all-cause death due to the range of pathophysiology (i.e., aging, renal function, and myocardial stress) reflected by elevated levels.

4.2 | NT-proBNP as a predictor for VAs and SCD

Although no specific mechanisms have linked NT-proBNP directly with risk of VA and SCD, myocardial stretch, which is the main

TABLE 3 Association between NT-proBNP and the risk of VA, hospitalization for HF, and all-cause mortality in patients with a primary prevention ICD indication and a secondary prevention ICD indication.

	C-statistics Harrell's C (95% CI)	Cox regression – Unadjusted model HR (95% CI)	*p*	Cox regression – Multivariable model 1[a] HR (95% CI)	*p*	Cox regression – Multivariable model 2[b] HR (95% CI)	*p*
Primary prevention ICD indication							
VA (*n* = 60)	0.55 (0.47–0.63)	1.24 (1.02–1.51)	.03	1.24 (1.01–1.52)	.04	1.10 (0.86–1.40)	.46
HF Hospitalization (*n* = 54)	0.81 (0.75–0.87)	3.07 (2.27–4.14)	<.001	3.25 (2.39–4.43)	<.001	3.23 (2.28–4.57)	<.001
All-cause mortality (*n* = 46)	0.81 (0.74–0.87)	2.51 (1.91–3.30)	<.001	2.61 (1.87–3.64)	<.001	2.12 (1.46–3.07)	<.001
Secondary prevention ICD indication							
VA (*n* = 77)	0.71 (0.64–0.77)	1.59 (1.34–1.88)	<.001	1.53 (1.27–1.85)	<.001	1.32 (1.02–1.70)	.04
HF hospitalization (*n* = 33)	0.88 (0.83–0.94)	3.12 (2.33–4.18)	<.001	3.56 (2.45–5.18)	<.001	3.49 (2.03–5.99)	<.001
All-cause mortality (*n* = 30)	0.83 (0.75–0.90)	2.42 (1.80–3.26)	<.001	2.09 (1.46–3.01)	<.001	1.90 (1.16–3.10)	.01

Note: Analyzed by proportional Cox regression per log unit increase of NT-proBNP in association to events in unadjusted model and after adjustments for risk factors in two separate models. Also presented is Harrell's C-statistics for the unadjusted model.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; GFR, glomerular filtration rate; HF, heart failure; LVEF, left ventricular ejection fraction; VA, ventricular arrhythmia.

[a]Adjusted for age, sex and BMI.

[b]Adjusted for age, sex, BMI, CAD, HF, estimated GFR, and LVEF.

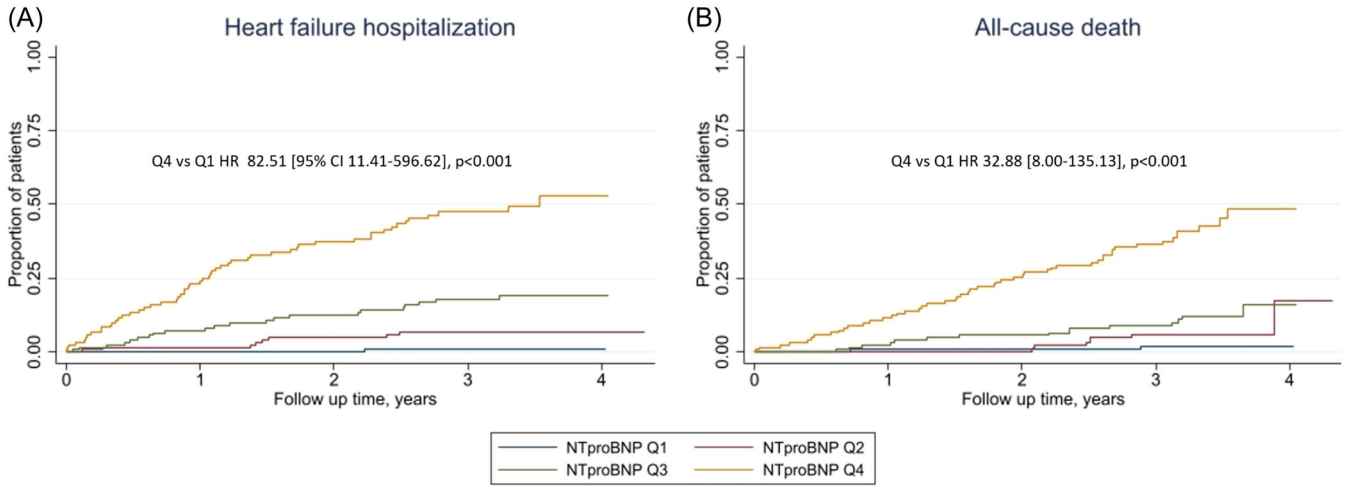

FIGURE 2 Association between baseline concentrations of N-terminal-pro B-type natriuretic peptide (NT-proBNP) and time to (A) heart failure hospitalization and (B) all-cause death in the total population. Stratified by quartiles of NT-proBNP and *p* is for Quartile 4 versus Quartile 1.

stimulus for the synthesis and secretion of natriuretic peptides,[19] have been proposed as a potential arrhythmic trigger.[7,11,20] Myocardial stretch can trigger mechano–electrical feedback leading to complex electrophysiological disturbances that can enhance different arrhythmogenic processes, triggering automaticity, triggered activity and reentry.[21–23] Previous studies have suggested that NT-proBNP can help predict SCD,[6–9] however, with different definitions of SCD. The majority of studies in this field define SCD as sudden and unexpected death, presumed to be arrhythmic occurring within 1 h of onset of symptoms, or if the deceased has been witnessed to be stable within 24 h of the arrest in case of unwitnessed death.[6,8,10,24] Diverging and vague definitions of SCD are unfortunate limitations of many of the published studies as it does not rule out other nonarrhythmic sudden death etiologies. An important strength of our study is that we included patients with implanted ICD with the advantage

of having documented arrhythmias with relatively long follow-up. We also analyzed NT-proBNP in one batch from blood samples stored in a dedicated biobank, which reduces the risk of analytical bias. In concordance with other studies of patients with ICD our results demonstrate a significant association between NT-proBNP and incident VA.[25–27]

In a meta-analysis, LVEF was demonstrated to not influence the association between natriuretic peptides and SCD in patients with and without ICD.[7] Our findings support that the association between elevated NT-proBNP and VA is independent of LVEF.

4.3 | NT-proBNP in primary versus secondary prevention

Our results suggest that there was an interaction by ICD indication on the association between NT-proBNP and risk of VA. The ability to discriminate between patients with and without incident VA was stronger in patients with a secondary ICD-indication, and for these patients the association persisted in adjusted models. In patients with a secondary prevention ICD indication, 11% in the lowest quartile of NT-proBNP had incident VA, whereas 52% had incident VA in the highest quartile. Guidelines recommend ICD implantation in patients who have experienced VA with hemodynamic consequences or within 48 h after myocardial infarction, in the absence of reversible causes. [28] However, there may be uncertainties related to whether the cause of VA is reversible and many patients with a low-risk of recurrent events never experience subsequent events. In these settings, our data support that NT-proBNP measurement may be helpful in assessing the risk of future VA, although this should be validated in future prospective cohorts.

Among patients with a primary ICD indication in our study, the performance of NT-proBNP in predicting VA was limited. Potential explanation for this may be that patients with advanced HF have non-arrhythmic mechanisms driving NT-proBNP secretions, such as neurohormonal activation and renal dysfunction.[29,30] This is supported by our finding of a strong association between NT-proBNP and the risk of all-cause death and HF hospitalization in these patients. Thus, our findings suggest that it is challenging to use NT-proBNP as a marker specifically for VA risk in patients considered for primary prevention ICD.

4.4 | Change in NT-proBNP from baseline to follow-up visit

There were no significant changes in NT-proBNP concentrations from baseline to the follow-up visit. Moreover, we found no association between the change in NT-proBNP concentration and the risk of subsequent incident VA. Although serial measurements of NT-proBNP may be useful in assessing HF status, our findings argue against repeated measurements for the purpose of arrhythmic risk stratification.

4.4.1 | Study limitations

Our cohort consisted of patients treated with ICD with high arrhythmic risk, and whether our results are applicable to other patient population is uncertain. The majority of patients in our study were men, which also is the case in similar cohorts. Women have intrinsically higher levels of NT-proBNP than men, and whether the results can be generalized to women is less certain. However, in the Nurses' Health Study, NT-proBNP was associated with the risk of SCD in 121 700 women.[24] Analytical variability of NT-proBNP measurements may be a reason for bias, which we tried to overcome by analyzing all samples in one batch using the same assay and instruments. Survival bias may have been introduced for the analysis using serial sampling, as death was the most important reason for nonattendance at the follow-up visit. The analysis stratified for ICD indication was posthoc and with limited power and must therefore be considered hypothesis-generating.

5 | CONCLUSIONS

In our cohort of patients with ICD, we found a significant association between high NT-proBNP concentrations and the risk of developing VA, as well as HF hospitalization and death, independent of established risk factors. NT-proBNP is a noninvasive test that is widely available and reproducible. Our data suggest that NT-proBNP may be a helpful tool for assessing VA risk, particularly in patients with a secondary ICD indication.

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CONFLICT OF INTEREST STATEMENT

H. Røsjø has received personal fees from Novartis and Thermo Fischer BRAMHS, CardiNor, and SpinChip Diagnostics. T. Omland has served on advisory boards for Abbott Diagnostics, Roche Diagnostics, and Bayer, and has received research support from Abbott Diagnostics, Novartis, Roche Diagnostics, Singulex, and SomaLogic via Akershus University Hospital, and speaker's or consulting honoraria from Roche Diagnostics, Siemens Healthineers and CardiNor. P. L. Myhre has served on advisory boards and received speaker fees from Amarin, AmGen, AstraZeneca, Bayer, Boehringer Ingelheim, Novartis, and Novo Nordisk. All other authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplementary Material

to

N-terminal pro-B-type natriuretic peptide for prediction of ventricular arrhythmias; data from the SMASH Study

Supplemental Table 1 Baseline characteristics of the study population according to the ICD indication

	Primary prevention		Secondary prevention (n=240)	P-value for primary vs secondary
	HF indication (n= 225)	Other indications (n=25)		
Age, y	67.6 ± 10.8	57.2 ± 16.6	65.7 ± 12.9	0.45
Male sex	194 (86.6%)	18 (72.0%)	195 (81.2%)	0.25
Body mass index, kg/m2	27.8 ± 5.4	27.0 ± 3.3	27.6 ± 4.2	0.77
Systolic blood pressure, mmHg	121 ± 20	127 ± 18	129 ± 20	<0.001
Diabetes mellitus	50 (22.2%)	2 (8.0 %)	42 (17.5%)	0.35
Coronary artery disease	146 (65.5%)	6 (24.0%)	157 (66.8%)	0.21
Previous acute myocardial infarction	126 (56.8%)	2 (8.0 %)	148 (61.7%)	0.03
Cardiomyopathy	4 (1.8 %)	17 (68.0%)	13 (5.4 %)	0.19
Heart failure	225 (100.0%)	0 (0.0%)	168 (70.4%)	<0.001
Left ventricular ejection fraction, %	35 ± 11	57 ± 6	44 ± 12	<0.001
New York Heart Association class III-IV	29 (12.9%)	1 (4.0 %)	22 (9.2 %)	0.31
Previous documentation of ventricular arrhythmia	57 (25.6%)	7 (28.0%)	218 (90.8%)	<0.001
Estimated glomerular filtration rate, ml/min/1.73 m^2	69 ± 24	97 ± 28	75 ± 23	0.14
NT-proBNP, ng/L	831 (288-1920)	169 (116-516)	442 (192-1058)	<0.001

Supplemental Table 2. Predictors of baseline NT-proBNP levels assessed by multivariable logistic regression models including all variables in the table.

	Beta coefficient [95% CI]	Z-value	P-value
Age	0.01 [0.004-0.02]	2.8	0.005
Male sex	-0.22 [-0.50-0.05]	-1.6	0.11
Body mass index, kg/m2	-0.45 [-0.67- -0.24]	-4.2	<0.001
Systolic blood pressure, mmHg	-0.001 [-0.051- -0.053]	0.03	0.98
Diabetes mellitus	0.26 [-0.01-0.51]	2.0	0.05
Previous acute myocardial infarction	0.17 [-0.05-0.40]	1.4	0.17
Heart failure	0.49 [0.16-0.82]	3.0	0.003
Left ventricular ejection fraction, %	-0.03 [-0.04- -0.02]	-6.7	<0.001
New York Heart Association class III-IV	0.37 [0.06-0.70]	2.3	0.02
Cardiomyopathy	0.71 [0.24-1.17]	3.0	0.003
Previous documentation of ventricular arrhythmias	0.05 [-0.15-0.26]	0.6	0.54
Estimated glomerular filtration rate, ml/min/1.73 m^2	-0.01[-0.02—0.01]	-5.6	<0.001

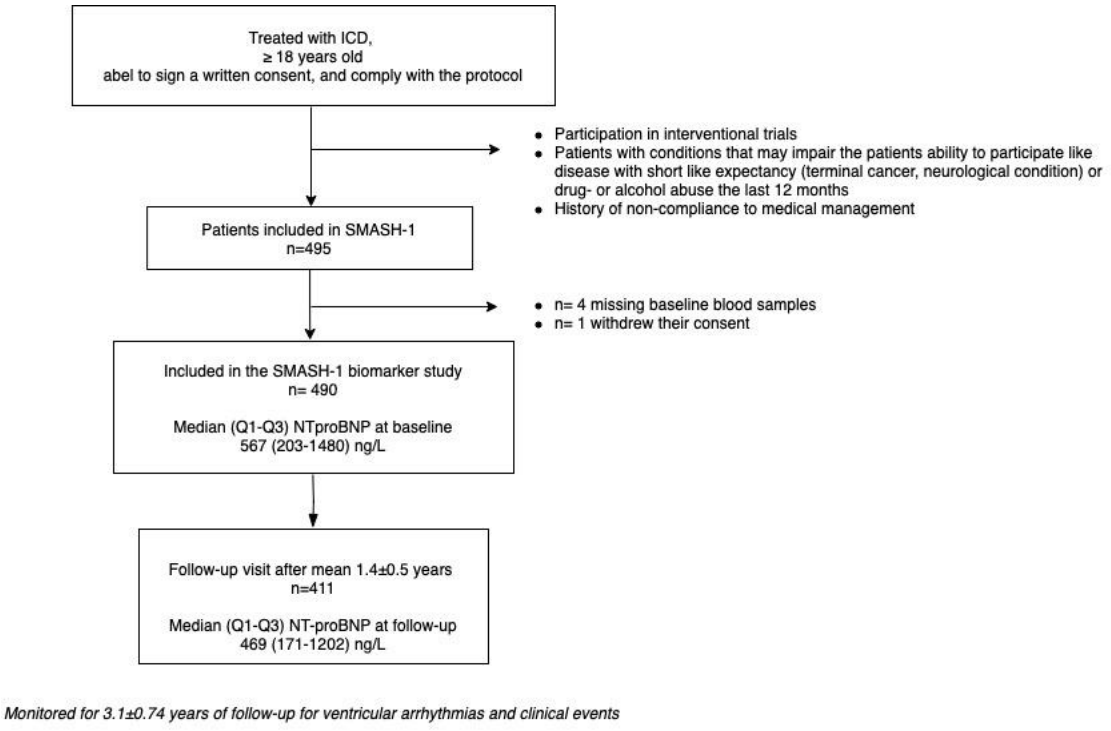
Suppl. Table 3 Baseline characteristics of the study population in patients with available blood samples at the follow-up visit and those without.

	Available blood samples at the follow-up visit *n=411*	**Not available blood samples at the follow-up visit** *n=79*	**P-value**
Age, y	65.9 ± 12.2	67.1 ± 13.3	0.45
Male sex	339 (82.5%)	69 (87.2%)	0.31
Body mass index, kg/m2	28.0 ± 4.8	26.1 ± 4.1	0.002
Systolic blood pressure, mmHg	125 ± 20	126 ± 23	0.63
Diabetes mellitus	72 (17.5%)	22 (27.8%)	0.03
Coronary artery disease	259 (64.0%)	50 (64.1%)	0.98
Previous acute myocardial infarction	227 (55.6%)	49 (62.0%)	0.29
Heart failure	324 (78.8%)	70 (88.6%)	0.05
Left ventricular ejection fraction, %	41 ± 13	38 ± 12	0.03
New York Heart Association class III-IV	37 (9.0 %)	15 (19.0%)	0.01
Cardiomyopathy	29 (7.1 %)	5 (6.4 %)	0.84
Previous documentation of ventricular arrhythmias	240 58.5%)	42 (53.8%)	0.44
Primary ICD indication	204 (49.6%)	46 (58.2%)	0.16
Estimated glomerular filtration rate, ml/min/1.73 m^2	74.6 ± 22.7	68.4 ± 29.7	0.04
Baseline NT-proBNP, ng/L	511 (178-1313)	1248 (524-2858)	<0.001

Suppl. Table 4 Baseline characteristics by quartiles of relative change in NT-proBNP from baseline to the follow-up visit

	NTproBNP Change Q1 n=103	NTproBNP Change Q2 n=103	NTproBNP Change Q3 n=103	NTproBNP Change Q4 n=102	P-value for trend
Relative change in NT-proBNP (median Q1-Q3)	*-50% (-60% to -43%)*	*-18% (-26% to -10%)*	*12% (6% to 24%)*	*84% (60% to 141%)*	
Age, y	65.1 ± 13.0	65.0 ± 12.0	66.5 ± 11.6	67.2 ± 12.2	0.14
Male sex	81 (78.6%)	85 (82.5%)	85 (82.5%)	88 (86.3%)	0.17
Body mass index, kg/m2	28.8 ± 5.5	27.8 ± 4.3	27.7 ± 4.6	27.6 ± 4.5	0.07
Systolic blood pressure, mmHg	122 ± 20	124 ± 16	126 ± 21	127 ± 21	0.046
Diabetes mellitus	21 (20.4%)	13 (12.6%)	16 (15.5%)	22 (21.6%)	0.71
Coronary artery disease	60 (59.4%)	66 (64.7%)	74 (72.5%)	59 (59.0%)	0.75
Previous acute myocardial infarction	53 (52.0%)	57 (55.3%)	65 (64.4%)	52 (51.0%)	0.79
History of heart failure	84 (81.6%)	81 (78.6%)	84 (81.6%)	75 (73.5%)	0.24
Left ventricular ejection fraction, %	39 ± 12	40 ± 14	40 ± 13	44 ± 12	0.005
New York Heart Association class III-IV	6 (5.8 %)	8 (7.8 %)	15 (14.6%)	8 (7.8 %)	0.31
Cardiomyopathy	7 (6.8 %)	10 (9.7 %)	7 (6.8 %)	5 (4.9 %)	0.45
Previous documentation of ventricular arrhythmias	58 (56.3%)	57 (55.3%)	60 (58.3%)	65 (64.4%)	0.22
Primary ICD indication	58 (56.3%)	53 (51.5%)	50 (48.5%)	43 (42.2%)	0.04
Estimated glomerular filtration rate, ml/min/1.73 m^2	74.6 ± 22.5	74.4 ± 21.9	77.9 ± 24.6	71.5 ± 21.6	0.58

Supplemental Figure 1. Flow diagram of the SMASH 1 biomarker study



III

Paper III