Faculty of Health Sciences,
University of Copenhagen

Ph.D. thesis

Electrocardiographic Monitoring and Cardiac Computed Tomography Angiography to Evaluate the Risk of Stroke

Identification of risk variables for ischemic stroke

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The Faculty of Health Science, Copenhagen University, Denmark has approved this PhD dissertation for public defense. The public lecture and defense will take place April 2nd, 2012 at 14.00 in Auditorium of Medical Museion, Bredgade 62, DK-1260 Copenhagen K.

Preface

This thesis is based on some of the follow-up results of the Copenhagen Holter study, a random epidemiological survey, and on the cardiologic aspects of a prospective cohort study where patient admitted with neurological deficits and suspected for having ischemic stroke/TIA at the Copenhagen University Hospitals of Amager and Bispebjerg. I wish to thank all patients who participated in both studies and made this thesis possible.

The Copenhagen Holter study was supported by the Danish Heart Foundation and part of this ph.d thesis was funded by grants from the Jens Anker Andersen Foundation.

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Zeynep Binici,
February 2011

Xastina min ji bo herkesran azadi, jiyan bi serfirazi û dilxesi, sefqat, xwesi û selameti. Zêvê, kecikan Heme Rasho.

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List of Papers

This Ph.D. thesis is based on the following two papers and one prospective study:

1 Binici Z, Intzilakis T, Nielsen OW, Køber L, Sajadieh A. Excessive supraventricular ectopic activity and increased risk of atrial fibrillation and stroke. *Circulation* 2010; 121:1904-1911.

2 Binici Z, Mouridsen MR, Køber L, Sajadieh A. Decreased Nighttime Heart Rate Variability is Associated with Stroke Risk. Stroke 2011;42:3196-3201.

3 Descriptive study of patients admitted with stroke focusing on silent arrhythmias and coronary artery disease.

Abbreviations

ACE-I	Angiotensin-Converting Enzyme inhibitor
AF	Atrial fibrillation
AHA	American Heart Association
AMH	Copenhagen University Hospital of Amager
BBH	Copenhagen University Hospital of Bispebjerg
BMI	Body Mass Index
CAD	Coronary artery disease
CES	Cardioembolism
CHS	Copenhagen Holter Study
CI	Confidence Interval
CTA	Computed Tomography Angiography
CT	Computed Tomography
DBP	Diastolic blood pressure
ECG	Electrocardiography
ESVEA	Excessive supraventricular ectopic activity
HbA_{1c}	Hemoglobin A_{1c}
HR	Hazard Ratio
HRV	Heart rate variability
hs-CRP	*high sensitive*-C-Reactive Protein
IHD	Ischemic heart disease
LAA	Large-artery atherosclerosis
LMS	left main stem stenosis
LVEF	Left ventricular ejection fraction
MeanNN	Mean of normal RR (NN) interval
MI	Myocardial infarction
MRI	Magnetic Resonance Imaging
mRS	modified Rankin Scale
MSCT	Multisclice Computed Tomography
NT-proBNP	N-terminal B-type natriuretic peptide
PAF	Paroxysmal atrial fibrillation
rt-PA	Recombinant tissue plasminogen activator
SBP	Systolic blood pressure
SDNN	Standard deviation of normal RR (NN) interval
SMI	Silent myocardial ischemia
SVEA	Supraventricular ectopic activity
SVEC	Supraventricular ectopic complex
SVD	Small-vessel disease
TIA	Transient ischemic attack
TCI	Transient cerebral ischemic
TOAST	Trial of Org 10172 in Acute Stroke Treatment
UND	Unknown Cause despite diagnostic efforts
VD	Vessel disease
VPC	Ventricular premature complexes

1. Introduction

Stroke: definition, prevalence, incidence, subdivision, and prognosis

Stroke is defined as neurological deficits lasting for more than 24 hours. Paralysis and reduced linguistic, perceptual and intellectual disabilities as well as disturbance of emotions are common and serious consequences, resulting in major personality changes, whereby normal life and family life is reduced or terminated[1;2]. TIA (Transient Ischemic Attack) has the same symptoms but with remission within 24 hours. According to guidelines from AHA (American Heart Association) distinction between ischemic stroke and TIA is not important because they share the same etiology, pathology and risk profiles[3].

Stroke is the third leading cause of death and the leading cause of disability in Europe, the United States, and many other countries in the world[4-9]. In Denmark (5.3 million inhabitants) stroke inflicts annually more than 15000 subjects[10]. In the United States, stroke costs about $30 billion per year in direct costs and loss of productivity[11]. In Scandinavian countries stroke consumes more hospital days than any other somatic disease[12-14]. Incidences of stroke-related deaths and disability are expected to rise even higher as the population ages[2]. More than half a million people in the United States experience a new or recurrent stroke each year[15].

Stroke kills about 150.000 Americans each year, and almost one out of three stroke victims, three million Americans, are currently permanently disabled from stroke.

Two-thirds of strokes occur in people over age 65[16]. Strokes affect men more often than women, although women are more likely to die from a stroke; strokes affect blacks more often than whites, and are more likely to be fatal among blacks[17-20]. These facts illuminate the significance of preventive measures to avoid stroke and stroke related mortality and morbidity.

Strokes are classified into ischemic and hemorrhagic subtypes, where the ischemic type constitutes about 85% of the cases[3;21;22]. Ischemic stroke is caused by occlusion of a cerebral artery either by atherosclerotic thrombi or emboli[23]. Several test methods are required in order to classify ischemic stroke in the acute phase. Tools, which are used for risk factor profiles are CT (computed tomography) or MRI (Magnetic Resonance Imaging) scanning, vascular imaging, such as Transcranial Doppler carotid duplex, ECG (electrocardiography) and echocardiography.

Classification of ischemic stroke is based on etiology. Subdivisions are based both on a history and clinical and neurological examination. Ischemic stroke is subdivided into five groups such as LAA (Large-artery atherosclerosis), CES (cardioembolism)[24;25], SVD (Small-vessel disease), UND (Unknown Cause despite diagnostic efforts) or other determined cause. According to this classification, called TOAST (Trial of Org 10172 in Acute Stroke Treatment), criteria for ischemic stroke can be used with advantage to differentiate between small-vessel diseases from other subtypes26. It is essential to classify ischemic stroke subtypes in particular with regard to prognosis27.

Cardioembolism stands for around 15-30% of all strokes of the ischemic type[25;28]. Atrial fibrillation contributes with approximate 15 to 25% of the cardioembolic sources. Small-vessel disease accounts for 25% and large-artery atherosclerosis for 15%. Approximately 15 percent is being classified as unknown etiology and the remaining 15 % are subtyped as other determined causes[29].

With regard to prognosis small-vessel disease has a better prognosis than other subtypes. Mortality rates are highest among patients with cardiogenic emboli[22]. One study points out that patient with small-vessel disease have 3 times the probability of being alive after two years than patients classified with cardioembolic stroke[29].

Risk factors for stroke

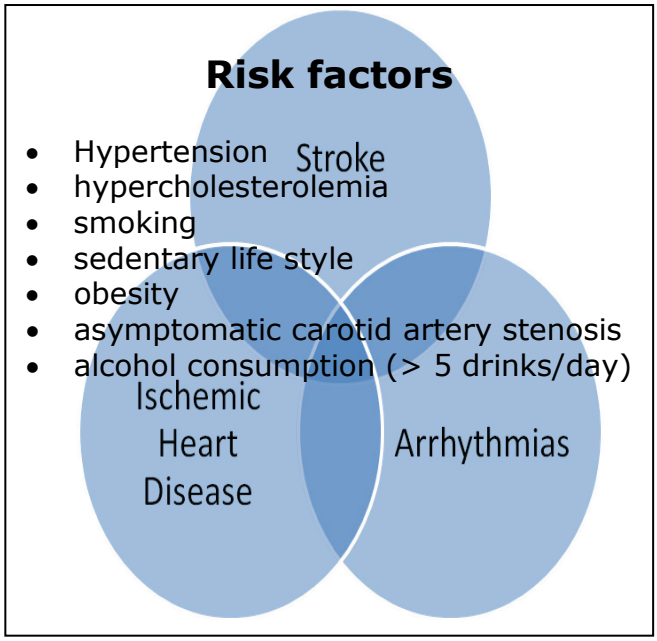
The most significant risk factors for stroke due to atherosclerotic thrombi are hypertension, hypercholesterolemia, diabetes mellitus, smoking, sedentary life style, obesity, asymptomatic carotid artery stenosis, excessive alcohol consumption (> 5 drinks/day)[30] (Figure 1). Major risk factors for cardio embolic stroke are atrial fibrillation, myocardial infarction (MI), congestive heart failure, valvular abnormalities, including prosthetic valves, and atrial septal defect[25].

Atrial fibrillation is increasing with age and becomes more common as the population age is increasing[31]. The detection of paroxysmal atrial fibrillation is not easy since many cases may be asymptomatic and it is not even apparent how much this condition really contributes to the incidence of stroke. One may expect that many cases of so-called “cryptogenic emboli” could be due to paroxysmal atrial fibrillation (PAF). Continuous electrocardiographic monitoring may be able to identify cases of PAF or other conditions that predispose to this condition and thus stroke[32].

Primary prevention strategies are based on modification of the identified modifiable risk factors[30;33;34]. Treatment of hypertension[35-39], statins for hypercholesterolemia[40;41], and antithrombotic or anticoagulation therapy for atrial fibrillation have been proved effective to reduce the rate of stroke in primary prevention context[42-45], while the use of aspirin or carotid endarterectomy for asymptomatic stenosis have not been proven effective[46].

The incidence of stroke is increasing in spite of the improvements in treatments of some risk factors like hyperlipidemia, and focus on hypertension treatment and other risk factor modification. Thus identification of the other significant risk factors for stroke is important to identify high-risk subjects for either interventional studies or targeted intensive risk modification[47].

Figure 1 - Association of stroke, ischemic heart disease and arrhythmias



Possible risk factors

Excessive supraventricular ectopic activity (ESVEA): While premature supraventricular complexes are seen in the majority of subjects studied, their contribution to stoke incidence has not been studied. Excessive number of these contractions may lead to electrical instability in the atrium and thereby atrial fibrillation/paroxysmal atrial fibrillation. ESVEA may also reflect structural abnormalities in the heart/atrium like fibrosis or hypertrophy either as a primary abnormality or secondary to other conditions like hypertension. In any case this may increase the risk of stroke. The possible association between ESVEA and stroke has not been studied before.

Reduced heart rate variability: The autonomic nervous system controls heart rate and heart rate variability reflects the influence of both the sympathetic and parasympathetic nervous system[48]. Several studies have revealed that autonomic dysfunction and reduced heart rate variability (HRV) are associated with increased mortality and cardiovascular disease[46,48]. A weak parasympathetic control may be responsible for increased risk of cardiovascular events[49]. Stroke survivors do have impaired function of the autonomic system and disturbances in sympathovagal balance may predict survival after the acute phase of stroke. Survival after stroke correlates with reduced heart rate variability[50;51].

It is believed that decreased vagal tone correlates with development of paroxysmal atrial fibrillation (PAF)[31;52]. A circadian variation in stroke onset among patients with atrial fibrillation have been demonstrated in one study[53].

In apparently healthy subjects, a reduced HRV may be a risk factor for stroke but this has not been studied properly.

Excessive ventricular ectopic activity: Excessive ventricular ectopic activity has been shown to be associated with sudden death, and increased cardiovascular mortality both in subjects with structural heart disease and in the general population[54-56]. In apparently healthy subjects the presence of excessive ventricular ectopic activity may be the first evidence of structural heart disease e.g. coronary heart disease, hypertensive heart disease of other conditions[57]. Thus an association with the risk of stroke could be possible.

Transient ST-segment depression: Transient ST-segment depression without any symptom is usually considered as silent myocardial ischemia (SMI). The risk of false positive finding exists and may be higher in "low-risk" subjects and lower in "high-risk" subjects (Beyes theorem). In apparently healthy subjects the observation SMI may reflect the possibility of significant silent atherosclerosis. This may be risk factor for stroke. Kurl et *al*. showed a significant association between exercise-induced SMI and risk of stroke[58].

Stroke and ischemic heart disease

Ischemic heart disease (IHD) is mainly due to coronary atherosclerosis and causes different symptom manifestations as stabile or unstabile angina, heart failure[59] or acute coronary syndrome. In Denmark ischemic heart disease is the leading cause of hospitalization and the second leading cause of mortality[60].

After MI stroke is highly prevalent regardless of type and it is one of the leading causes of long-term mortality[61]. SMI may be underestimated[62-65] and increases risk of mortality and stroke[66;67]. In addition, Magnetic Resonance Imaging studies reveal that asymptomatic strokes[68;69] are much more prevalent than symptomatic ones[69]. Clinically silent cases are often evident[68;70]. Coronary plaques occur in 72.4% and 37.5% have coronary stenoses in autopsies of patients with stroke[71]. By using coronary angiography, Amarenco et *al*. found a high burden of silent coronary disease in patients with nonfatal stroke and without known coronary heart disease: Almost 62% had plaques and 25.7% had significant stenosis[72].

Moreover it has been found that 40.8% of silent cases of MI in autopsies in subjects suffered from stroke, revealing a high prevalence of ischemic heart disease in subjects with stroke[73;74]. However, there have been only few studies evaluating coronary artery disease in subjects with stroke and no apparent heart disease[73;74]. During the course of acute stroke of ischemic type, ECG changes can be seen and may be of uncertain clinical relevance; however, coexisting ischemic heart disease may be a major issue[75]. Elevation in cardiac troponins in subjects with stroke has been reported previously[76]; in one study, about 16% of the 330 consecutive patients with stroke had elevation of cardiac troponin I[77]. Another study, 9.6% of the 279 patients with ischemic stroke had elevated troponin T levels[78]. Increased levels of troponins in these situations are also associated with a poor prognosis[77].

Using a 64-multislice CT scanning to creating 3D images of the heart, this non-invasive test is used to reveal whether fatty deposits or calcium deposits have built up in the coronary arteries[79-83]. A meta-analyses by Abdulla et *al.* revealed a sensitivity and specificity of 95.5 % and 81 % respectively compared to invasive angiography[84].

If left untreated, these areas of build-up, called plaques, can form basis for ischemic heart disease and in turn lead to either diastolic or systolic heart failure[85-87] with symptoms like fatigue dyspnoea, cardiomegalia, peripheral oedema, angina pectoris, cardiac arrhythmias or acute coronary syndrome. However, there was no systematic study evaluating coronary arteries by using a multislice CT-scanner in subjects with stroke at the time this PhD. study was launched.

2. Objectives

The objectives of this thesis are:

1.
By assessing data from the Copenhagen Holter Study to evaluate the electrocardiographic risk factors for development of stroke and atrial fibrillation in apparently healthy middle-aged and elderly subjects. The following baseline electrocardiographic abnormalities were focused on:
Supraventricular arrhythmias other than atrial fibrillation,
Heart rate variability,
Dynamic ST-segment depression (SMI),
Ventricular arrhythmias

Hypothesis: Both heart rate variability and supraventricular arrhythmias other than atrial fibrillation are predictors of future stroke.

2.
A. In a cohort of subjects with recent stroke and sinus rhythm to evaluate the rate of some relevant electrocardiographic abnormalities (ESVEA, excessive ventricular ectopic activity, and reduced HRV) compared with controls from Copenhagen Holter Study

B. To evaluate the prevalence of concomitant coronary artery disease in subjects with stroke without apparent or known heart disease. By using cardiac CTA and measuring coronary artery calcium content (score) and obtaining data on coronary artery stenoses in patients with stroke/TIA.

Hypothesis: Patients with recent stroke have more supraventricular ectopic activity, reduced heart rate variability and higher calcium score in the coronary arteries than age matched controls.

3. Identification of risk variables for ischemic stroke/TIA

3.1 Holter monitoring

Holter monitoring[88] is continuous ECG recording with a device that the person carries around and provides information about heart rate, heart rate variability, cardiac arrhythmias, supraventricular or ventricular ectopic activity or silent myocardial ischemia during normal activity[67]. Holter recordings are an easy non-invasive clinical tool, and can be quickly obtained. The Copenhagen Holter study aimed to address the value of 48-h Holter recording in risk assessment of middle-aged and elderly men and women with no apparent heart disease[89].

3.2 Methods

3.2.1 The Copenhagen Holter Study

The Copenhagen Holter study, a population-based prospective cohort study, is the largest Holter study in apparently healthy subjects and was carried out from 1998 to 2000. The details of the study protocol and selection procedures have been published previously[90;91]. Briefly, by using the national Danish Civil Registration System, all men aged 55, and all men and women aged 60, 65, 70 and 75 years (n=2969) within two well-defined postal regions in Copenhagen city were contacted and received a questionnaire asking about cardiovascular risk factors, use of medication and medical history. All participants who have accept the invitation had implemented a 48-hour Holter recording, fasting laboratory tests and a brief physical examination.

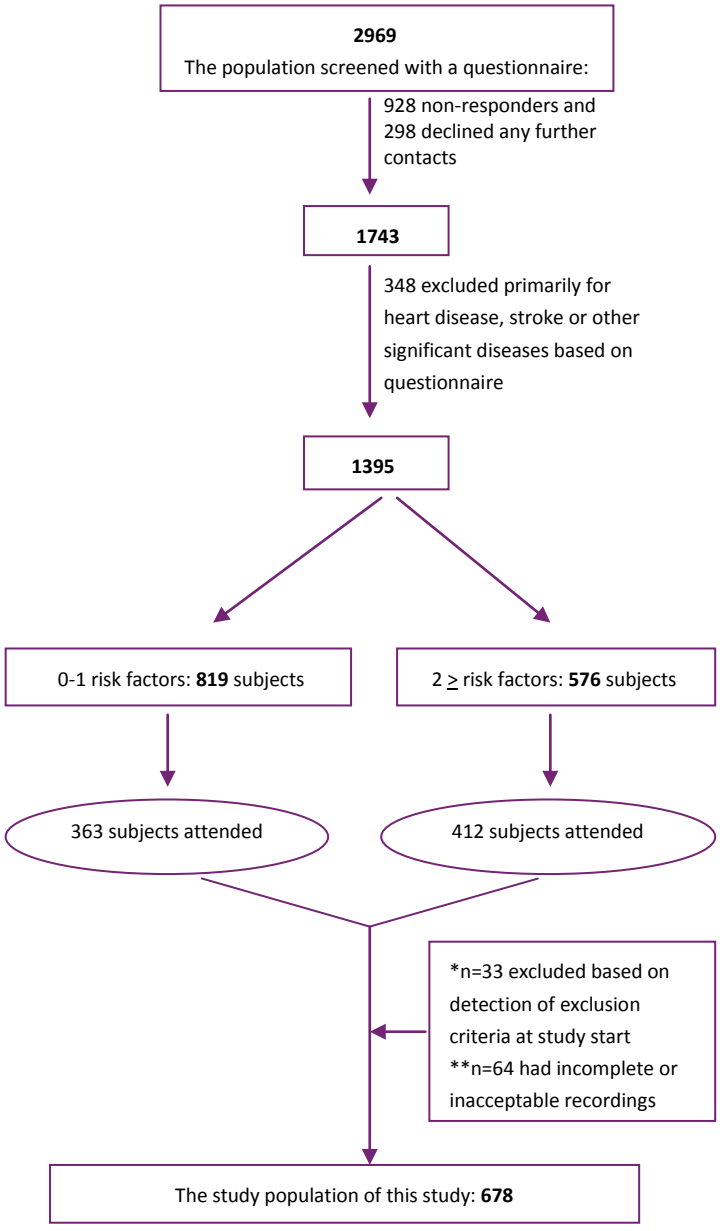
Subjects were ranked according to the number of the following risk factors: hypertension, diabetes mellitus, smoking, familial predisposition to cardiac disease, obesity and hypercholesterolemia. Exclusion criteria were known arrhythmic heart disease including permanent atrial fibrillation, manifest ischemic heart disease, angina pectoris, congestive heart disease, valvular heart disease, congenital heart disease, medical treatment for any heart disease, a history of stroke, cancer, other significant or life threatening diseases, abnormal Q-waves (I 1-3), left bundle branch block (VII-1), and ST depression $\geq$1 mm (IV-1) on baseline standard ECG. This study invited all subjects with two or more of the above mentioned risk factors, and a random sample consisting of 60% subjects with one or no apparent risk factor (Figure 2).

3.2.2. Holter Device

The study has up to 48-h successful Holter monitoring in 678 subjects. More than 98% of the study population had more than 24 hours recording.

Holter recording for up to 48 hours was carried out by the use of two-channel SpaceLabs tape recorders (9025, SpaceLabs, Inc., Redwood, WA, USA). From the 48-hour Holter recording, the first 24 hours were selected for analyses (the 2nd to the 25th hour). The primary editing and analyses were performed by experienced personnel and supervised by a responsible cardiologist. All Holter analyses were performed blinded to the other patient data. An analysis of HRV was done using an FT3000 Medical Analysis and Review Station. The quality of the analyses has previously been described in detail, and the interobserver variability shows kappa values between 0.91 and 0.94[92]. Reproducibility was tested by a blinded reanalysis of 50 tapes, and the Spearman's rank correlation coefficient between the measurements was 0.95 to 0.97. The range of technically acceptable recording and analysis time was 17.2 - 49.2 hours. The median value was 44.1 hours (Q1 and Q3: 41.4 - 45.5 hours).

Figure 2 - Flow diagram of inclusion steps



3.3. Event definitions and follow-up

The primary endpoint was the combined endpoint of first ever stroke event or all-cause mortality. Secondary endpoints were stroke, admissions for atrial fibrillation and all-cause mortality alone.

At follow-up time (median 76 months) data on stroke, atrial fibrillation and death was obtained through the national central patient registry. All deaths, hospital admissions and discharges in Denmark are reported to this registry within two weeks. The diagnosis of stroke was based on history and typical findings, i.e. neurological deficits and verification with either CT or MRI scanning of cerebrum. In case of registration with a diagnosis of stroke, death and atrial fibrillation, all discharge letters and hospital records were evaluated. All cases of stroke were needed to be adequately documented (clinical, CT-scanning, MRI scanning). Al strokes in this thesis were of ischemic type. Hemorrhagic stroke (intracerebral hemorrhage and subarachnoidal hemorrhage) were excluded from this study. Medicine use at baseline was obtained and no participants were on treatment with anticoagulants.

3.4. Definitions

A. Supraventricular arrhythmias other than atrial fibrillation. Two classes of supraventricular arrhythmias were studied.

- Isolated supraventricular ectopic complexes (SVEC).
- Runs of three or more SVEC.

Identification of SVEC was based on three criteria: prematurity, post contraction pause, and morphology. The morphology in this regard means QRS duration. A QRS with a duration less than or equal to 0.11 sec was accepted as a supraventricular complex without any regard to the following pause. A complex wider than 0.11 sec and with a compensatory pause was usually accepted as ventricular, even though some supraventricular complexes may behave like this.

Coupling interval to preceding QRS complex had to be 70% or less of mean RR interval of basic rhythm prior to the event. QRS complexes had duration of < 0.11 s unless aberration was suspected. The generally accepted methods to distinguish aberration: If the first part of the ectopic and widened complex has the same direction of the normal complex, then aberration were considered. If the following pause in these cases also were non-compensatory then complex were accepted as supraventricular complex. Post contraction pause had to be non-compensatory. The rhythm abnormalities are illustrated in Figure 3.

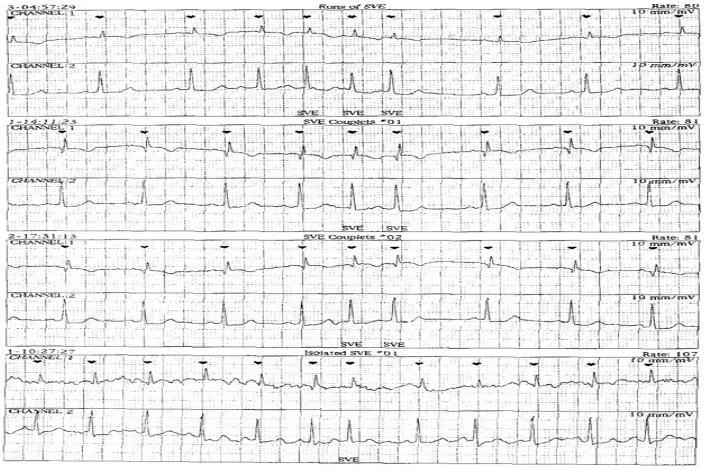
Frequency of SVEC and length of runs of SVEC were analyzed as both continuous and dichotomized variables. With the assumption that SVEC had to be excessive to increase the adverse events substantially, the cut-off value was set at the top 10 percentile for both frequency of SVEC and length of runs of SVEC. As follows, excessive supraventricular ectopic activity (ESVEA) was defined as ≥30 SVEC per hour or any episode of runs of ≥20 SVEC. Runs of SVEC were classified as "runs" and no distinction was made between short bursts of atrial fibrillation or ectopic atrial tachycardia.

B. Heart rate variability (HRV): HRV reflects fluctuation in heart rate over time, i.e. from beat to beat and over a longer time period and can be expressed by many different methods. In this study SDNN was chosen. SDNN is widely used and is the simplest time domain measures of HRV, and is defined as the standard deviation of normal to normal (NN) QRS intervals[93]. The association between HRV and stroke is not well studied. HRV is influenced by the autonomic nervous system which in turn might be influenced by daytime activities. Nighttime HRV might be freed from like incidental physical activity and mental stress. Hence it is assumed that nighttime HRV might be more a reproducible variable than 24-h HRV estimates.

Nighttime SDNN is calculated over a period of 15 min from 02:00 am to 02:15 am. 24-h SDNN is calculated for an entire 24-hour period.

For mean heart rate, we used MeanNN which stands for the mean value for the time between normal complexes. Nighttime MeanNN is analyzed during a sequence of between 2:00 am and 2:15 am. 24-h MeanNN is calculated for 24-h period. MeanNN for each period reflects the mean heart rate during that period (60.000 / MeanNN = heart rate in beat/ min).

Figure 3 - An example of rhythm abnormalities



C. Transient ST-segment depression: Analysis of ST- segment depression was performed semi-manually by trained personal at the Holter laboratory. An episode of ischemia was defined by a downsloped or horizontal ST depression of at least 1 mm, measured 60 msec. after the J-point of the QRS complex, and for at least one minute of duration, and separated from another episode by at least one minute of no ST-depression. The episodes of SMI were detected by the computer program and were evaluated by the technicians, who decided to accept or discard the episode, and additionally accepted or changed the suggested level of ST-depression by machine. Evaluation of the Holter recordings were completed before follow-up studies and performed by subjects blinded to and without any access to the participant's files.

D. Ventricular ectopic activity: Ventricular ectopic activity at different frequencies can be detected in the majority of cases studied. Several studies show that an excessive rate may be associated with increased risk of cardiovascular mortality or morbidity. Ventricular ectopic complexes are also associated with increased risk of adverse events at comparable frequencies[94;95]. The only ventricular arrhythmia that was assessed in this study was increased ventricular ectopic activity: > 30 ventricular premature complexes (VPC)/hours. Other ventricular arrhythmias like "runs" and "doublets" are strongly associated with excessive ventricular ectopic

activity and have prognostic impact in the context of excessive ventricular ectopic activity.

3.5. Statistical analyses

Statistical analyses were made by using SAS statistical software program (version 9.1, SAS Institute Inc., Cary, North Carolina, USA) and STATA/IC for Windows release 10 (College Station, Texas, USA).

Parametric and non-parametric tests were used to evaluate univariate associations between variables of interest. For the parametric tests to be used the following assumptions should be met: the distributional requirements of normality, homogeneity and linearity. Otherwise non-parametric tests were used to evaluate the differences between groups. Normal distribution was tested visually by probability plot or histograms. Not normally distributed variables were transformed or categorized if necessary. For normally distributed variables, the mean and standard deviation (SD) are presented and for not normally distributed variables median value and quartiles (Q1-Q3) are presented.

Unadjusted associations between variables of interest and various baseline parameters were evaluated by among other tests, Spearman's rank correlation, Kruskal-Wallis rank test, or $\chi 2$ test, Wilcoxon rank sum and paired t-test as appropriate.

Significance level with P values less than 0.05 were considered statistically significant.

To evaluate the adjusted associations, associated parameters with a p-value <0.05 were identified and examined in multivariate linear regression or multivariate logistic regression models with forced entry of age and sex. In forward selection or backward exclusion models $p \leq 0.05$ was used to enter the model and $p \leq 0.05$ to stay in the model.

Event free survival in groups of interest was evaluated by the method of Kaplan-Meier and differences were compared by means of the log-rank test.

Cox proportional hazard models were used to evaluate the confounder-adjusted associations with events. Conditions for Cox models, i.e. model assumptions – linearity of continuous variables, the proportional-hazard assumption, and lack of interactions – were tested and found to be valid unless otherwise indicated. Multifactorially adjusted models were adjusted for covariates of interest. Multivariable adjustments were performed primarily both for age and gender alone or together with other conventional risk factors: smoking, diabetes mellitus, systolic blood pressure and total cholesterol. The selection of the potentially confounding covariates was based on existing knowledge about their potential relationship with the variable in focus.

Since our studies were longitudinal studies the time scale had to be made use of.

Patient-year estimates were calculated to illustrate the event rates in groups of interest.

Two-tailed tests of significance were performed in spite of our hypothesis was to be one-tailed. This is common and recommended to be used routinely[96], as results could potentially go another way than believed.

For reasons of numerical stability of the estimates derived from a Cox model, there is a guideline that one should estimate no more parameters (i.e., beta coefficients) than the number of events divided by 10. This 'rule of 10' has in general been followed in order to avoid over fitting, and destabilization of the mathematical models. However, with caution it is possible to evaluate for further covariates[97]. We have tested all of our models in univariate models, and then in models with maximum one covariate per 10 events. Further adjustments with more variables were only accepted if the stability of the model could be shown. We reduced all models to only one variable per 10 events and then adjustments for more desirable variables were performed in further adjustment.

4. Results

Totally, 678 subjects participated to the Copenhagen Holter Study. The study population was 64.5 ± 6.8 years old and 41.2% were women. About 11.1% had diabetes and the average systolic blood pressure was 156.4 ± 24.2. Follow-up time was 74-78 months (Q1 to Q3) and the median is 76 months. More than 98% of the study population had more than 24 hours of recording. ESVEA and SMI were available for study in all study subjects whereas nighttime HRV was available for study in 653 subjects (96%).

4.1. Arrhythmias

4.1.1. Paper 1

Ninety-nine study subjects were identified with ESVEA, 70 subjects had more than 30 SVEC/h and 42 had runs of SVEC with a length of ≥20 SVEC, i.e. 13 subjects had both abnormalities. Events rates are shown in Table 1.

SVEC was analyzed both as dichotomized and continuous variable for the predefined primary endpoint and for all-cause mortality, stroke alone, and atrial fibrillation.

Table 1 – Total number of events and rate of events per 1000 patient-years (*in cursive*)

ENDPOINTS	N=678
Atrial fibrillation, n	22 *(5.5)*
Stroke, n	27 *(6.7)*
Total Mortality, n	87 *(21.4)*

Primary endpoint: The primary endpoint of death or stroke occurred in 105 of all participants: 29 in 99 subject with ESVEA and 76 in 579 subjects without ESVEA (p<0.0001). When corrected for conventional risk factors in a Cox model, ESVEA was associated with an increased hazard ratio (HR), HR: 1.64, 95% CI: 1.03-2.60, p=0.036 (Table 2a). SVEC as a continuous variable and runs of SVEC as a continuous variable were also associated with the primary endpoint in both univariate analyses: (HR: 1.44, 95% CI: 1.21-1.71, p<0.0001, for SVEC, and HR: 1.12, 95% CI: 1.05-1.21, p=0.001 for runs of SVEC) and the corresponding multivariable analyses showing almost the same result as in primary analyses.

Table 2 a. Multivariate Cox Regression models showing the risk of "death or stroke" or "all cause mortality" in relation to SVEC, runs of SVEC and ESVEA

	Hazard Ratio (95% CI) p-value	Hazard Ratio (95% CI) p-value
Multivariable *	**Death or Stroke**	**All-cause Mortality**
SVEC (For each increment of 10 SVEC/hour)	1.25 (1.04-1.52) 0.021	1.27 (1.04-1.55) 0.019
Runs of SVEC (For lengthening of runs by 4 SVEC)	1.06 (0.98-1.14) 0.16	1.06 (0.97-1.15) 0.20
ESVEA	1.64 (1.03-2.60) 0.036	1.40 (0.83-2.36) 0.207

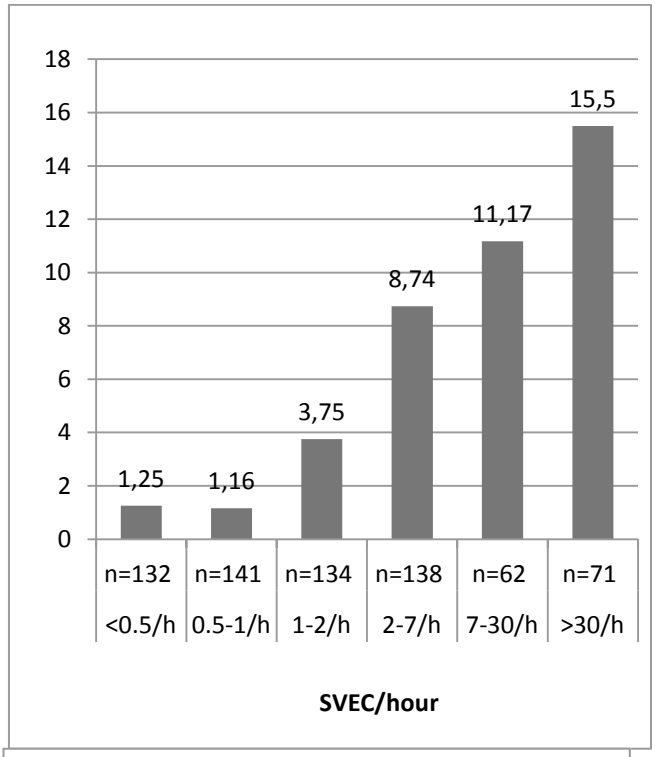
*Adjusted for age, gender, smoking, diabetes mellitus, systolic blood pressure, and total cholesterol.

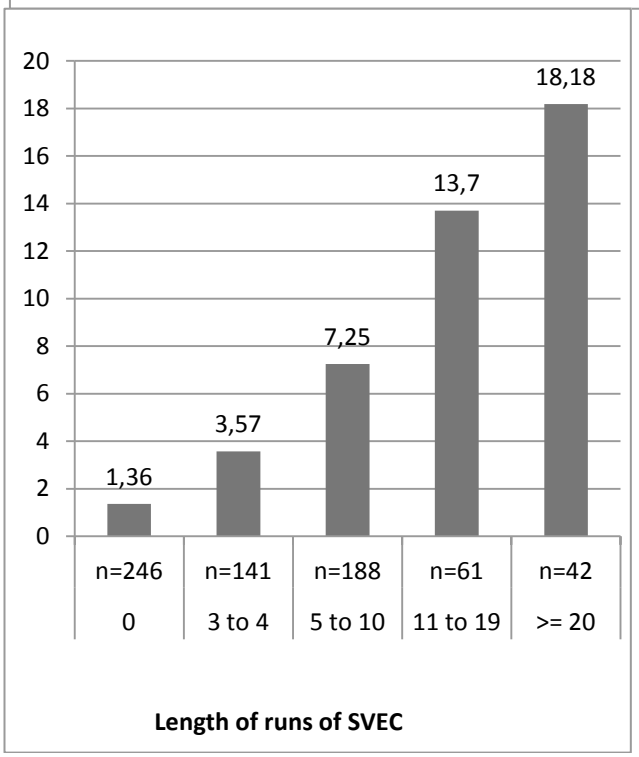
Stroke: The follow-up showed that 27 subjects experienced a stroke. Ten subjects with ESVEA experienced an event of stroke and subjects without ESVEA accounted for 17 cases (HR: 3.88, 95% CI: 1.78-8.48, p=0.0007). This result remained after adjustment for age and gender (HR: 2.79, 95% CI: 1.23-6.30, p=0.014) and after further adjustment for other major risk factors for stroke (HR: 2.37, 95% CI: 1.02-5.50, p=0.044). The association of stroke alone with ESVEA both as continuous and dichotomized variables was analyzed. ESVEA was not significantly associated with stroke as continuous variable but when used as a dichotomized variable, it demonstrated that subjects at higher frequencies of ESVEA had increased rate of stroke. The association between SVEC and stroke alone was not linear (data from Paper I).

Atrial fibrillation: The results showed strong associations between atrial fibrillation and SVEC/ESVEA both in univariate and also in multivariable analyse.

The association of SVEC with atrial fibrillation was evaluated, adjusted for the variables, which in univariate analyses were associated with atrial fibrillation. In contrast to stroke, which was more prevalent in patients with higher frequency of SVEC, a linear association between the frequency of SVEC or length of the runs of SVEC and incidence of atrial fibrillation was present (Figure 4). In this regard there was a factor of ten difference in the incidence of atrial fibrillation in subjects with highest frequency of SVEC versus those in the lowest groups (p=0.0006).

Figure 4 - Admissions of atrial fibrillation per 1000 patient-year (y-axis) in relation to the rate of SVEC and length of runs of SVEC





All-cause mortality: Twenty-one of the subjects with ESVEA and sixty-six of the subjects without ESVEA died during the follow-up. ESVEA was not associated with all-cause mortality in univariate analysis (Table 2b), but associated with all-cause mortality in multivariate analysis (Table 2a). SVEC as continuous variable was associated with total mortality in univariate as well as in multivariable analyses (Table 2a and 2b). Subjects without ESVEA had higher survival rates (the figure is shown in paper I).

Table 2 b. Univariate Cox models showing the risk of "death or stroke" or "all cause mortality" in relation to SVEC, runs of SVEC and ESVEA

	Hazard Ratio (95% CI) p-value	**Hazard Ratio (95% CI) p-value**
Univariate	**Death or Stroke**	**All-cause Mortality**
SVEC (For each increment of 10 SVEC/h)	1.44 (1.21-1.71) <0.0001	1.49 (1.24-1.79) <0.0001
Runs of SVEC (For lengthening of runs by 4 SVEC)	1.12 (1.05-1.21) 0.001	1.12 (1.03-1.21) 0.006
ESVEA	2.54 (1.66-3.90) <0.0001	2.12 (1.30-3.47) 0.003

Further analysis: In secondary analyses of the primary endpoint, with exclusion of the participant at the time of atrial fibrillation, the effect of atrial fibrillation on development of further events was assessed. These analyses showed that ESVEA was associated with primary endpoint both in unadjusted (HR: 2.63, 95% CI: 1.71-4.3, p<0.0001) and multivariable analyses (HR: 1.73, 95% CI: 1.09-2.75, p<0.020).

The results showed that SVEC as a continuous variable was significantly associated with the primary endpoint of stroke/mortality and atrial fibrillation but not for stroke alone when treated as a linear variable.

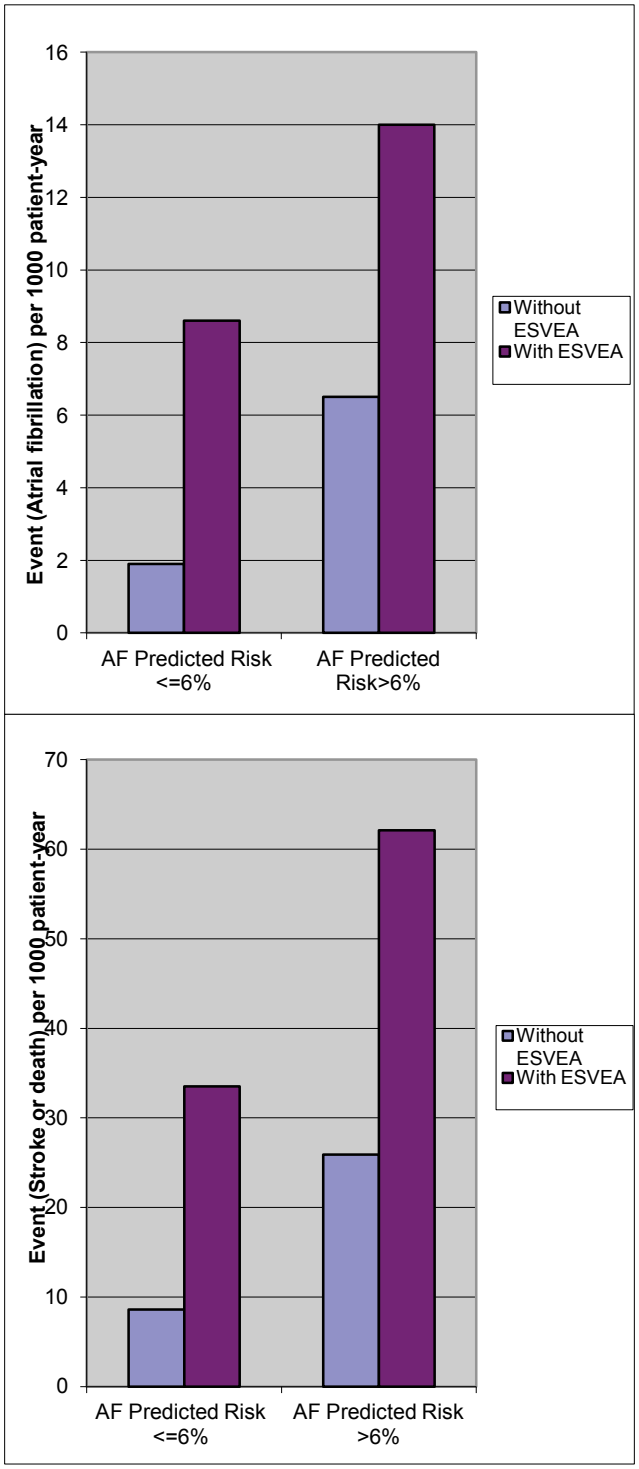
Further Risk Stratification

Modified Framingham atrial fibrillation risk score was calculated according to the method described by Schnabel et *al*.[98]. This method is based on information of age, gender, BMI, systolic blood pressure, hypertension, cardiac murmurs, PR distance on EKG, and information on heart failure. However, participants of this study did not have congestive heart failure and P-R interval was coded according to Minnesota codes. A calculated risk above 6% per 10-year, which was the median value, was used for risk stratification.

Stratification of the study population according to the Framingham risk score for atrial fibrillation above or below 6% per 10-year showed that risk of ESVEA was significantly higher in subjects with high-risk score (Figure 5). In Cox-regression models with inclusion of both SVEC and Framingham risk score for atrial fibrillation (both as continuous variables) SVEC remained significantly associated with atrial fibrillation (HR: 1.50, 95% CI 1.05-2.14, p=0.02).

Even in participants with low Framingham AF-risk ESVEA was able to identify subjects with relative high risk of atrial fibrillation. This signifies ESVEA's independency of this scoring and shows that ESVEA may give information beyond that performed by Framingham AF-risk score.

Figure 5 - Event rates in subjects with and without ESVEA stratified according to Framingham Risk Score for Atrial Fibrillation above or below 6%/10-year (p<0.0001 for both).



4.1.2. Paper 2

In these study 87 subjects died during the follow-up period, 26 subjects developed stroke and 20 subjects were admitted to hospital with atrial fibrillation. Eighty subjects were identified in the group of the combined endpoint of all-cause mortality and stroke.

Subjects in the lower half of nighttime SDNN compared to the rest of the study subjects were older (p=0.0539), had higher median systolic blood pressure (157 ± 25.1 mmHg versus 156 ± 23.5 mmHg) and had more diabetes (p=0.02). Haemoglobin A_{1c} (HbA_{1c}), triglyceride and *hs*-C-Reactive Protein (*hs*-CRP) levels were also higher compared to the subjects with higher nighttime values (p=0.047, p=0.04, p=0.022, respectively).

Nighttime SDNN was lower in women (39.5 ± 20 ms) than men (46.2 ± 25.1 ms) p<0.0001, and in diabetics (39.8 ± 23.9 ms) compared to non-diabetics (43.8 ± 23.2 ms), p=0.02. Triglyceride and *hs*-CRP were inversely related to nighttime SDNN, p=0.002 and p=0.004 respectively. However, no other covariate was associated with nighttime SDNN.

Nighttime SDNN was strongly correlated with death or stroke in univariate, age and gender adjusted and fully adjusted models. Similarly, 24-h SDNN, 24-h MeanNN and nocturnal MeanNN were also associated with the combined endpoint of death and stroke (Table 3a).

The risk of stroke was significantly associated with nighttime SDNN in univariate analysis, after adjustment for gender and age, and in a fully adjusted model.

However, nighttime MeanNN, 24h SDNN and 24h MeanNN were not associated with stroke in univariate or adjusted models (Table 3b). The association between nighttime SDNN and stroke remained significant even after further adjustment for heart rate, and significant biomarkers like triglyceride, *hs*-CRP and NT-pro BNP (HR: 0.96, 95% CI: 0.93-0.99, p=0.005).

Of the twenty-six subjects who experienced a stroke we found that 81% of all cases were in the lower half of nighttime SDNN reflecting a strong association with nighttime SDNN below this level (HR=4.31, 95% CI: 1.62-11.42, p=0.003).

Nighttime SDNN was associated with all-cause mortality in univariate analysis, and after adjustment for age and gender but not in fully adjusted models. However, 24h MeanNN, nighttime MeanNN and 24h SDNN were all strongly associated with all-cause mortality both in univariate and adjusted models (Table 3c).

Table 3a - Cox regression models for all-cause mortality and stroke as combined endpoind

	Nighttime SDNN (ms)	**Nighttime MeanNN (ms)**	**24-hour SDNN (ms)**	**24-hour MeanNN (ms)**
	Hazard Ratio (95% Confidence Intervals) p-value			
Univariate	0.836 (0.748-0.935) 0.002	0.696 (0.566-0.855) 0.001	0.682 (0.55-0.847) 0.001	0.769 (0.62-0.953) 0.016
Age and gender adjusted	0.83 (0.744-0.925) 0.001	0.6 (0.485-0.743) < 0.0001	0.652 (0.522-0.814) <0.0001	0.64 (0.51-0.803) <0.0001
Multivariable*	0.861 (0.774-0.956) 0.005	0.697 (0.554-0.877) 0.002	0.749 (0.597-0.94) 0.013	0.729 (0.577-0.923) 0.009

*Adjusted for age, gender, smoking, diabetes mellitus, systolic blood pressure, and total cholesterol.

Table 3b - Cox regression models for stroke as endpoint

	Nighttime SDNN (ms)	Nighttime MeanNN (ms)	24-hour SDNN (ms)	24-hour MeanNN (ms)
	Hazard Ratio (95% Confidence Intervals) p-value			
Univariate	0.669 (0.509-0.88) 0.004	0.853 (0.577-1.261) 0.425	0.768 (0.509-1.158) 0.207	0.892 (0.594-1.34) 0.583
Age and gender adjusted	0.666 (0.508-0.873) 0.003	0.751 (0.501-1.124) 0.164	0.734 (0.483-1.115) 0.147	0.769 (0.504-1.175) 0.225
Multivariable*	0.675 (0.513-0.888) 0.005	0.867 (0.566-1.33) 0.514	0.866 (0.563-1.333) 0.514	0.832 (0.535-1.3) 0.418

*Adjusted for age, gender, smoking, diabetes mellitus, systolic blood pressure, and total cholesterol.

Table 3c - Cox regression models with all-cause mortality as endpoint alone

	Nighttime SDNN (ms)	Nighttime MeanNN (ms)	24-hour SDNN (ms)	24-hour MeanNN (ms)
	Hazard Ratio (95% Confidence Intervals) p-value			
Univariate	0.881 (0.784-0.99) 0.033	0.68 (0.541-0.855) 0.001	0.661 (0.51-0.841) 0.001	0.767 (0.605-0.972) 0.028
Age and gender adjusted	0.875 (0.781-0.98) 0.02	0.587 (0.464-0.744) <0.0001	0629 (0.49-0.806) <0.0001	0.64 (0.498-0.823) 0.001
Multivariable*	0.911 (0.818-1.015) 0.092	0.686 (0.533-0.883) 0.003	0.723 (0.56-0.933) 0.013	0.738 (0.57-0.954) 0.021

*Adjusted for age, gender, smoking, diabetes mellitus, systolic blood pressure, and total cholesterol.

4.2. Silent Myocardial Ischemia

From all included study subjects from the Copenhagen Holter study with successful Holter recordings (n=678) twenty-seven persons developed stroke in the follow-up period and seventy-five persons were diagnosed with silent myocardial ischemia (SMI) according to the definition. From all participants with SMI 10.7% (8/75) developed a stroke during follow-up period as compared to a 3.2% (19/601) in subjects without SMI: HR 3.2 (95% CI 1.38-7.35, p=0.007) after adjustment for age and gender. Of all participants with SMI 26.7% (n=20) died or had stroke. Among persons without SMI (n=601) 14.14% (n=85) either died or developed stroke (P= 0.0069). Among all persons who developed stroke in the follow-up period 29.6% had SMI at baseline. Characteristics of the population at baseline are listed in table 4. Subjects with SMI were older than subjects without SMI and they had higher systolic blood pressure and BMI.

Sixty-three percent of all strokes (17/27) occurred in subjects with either SMI (n=77) or ESVEA (n=99); Hazard ratio (HR) for having one of these abnormalities was 5.90 (95% CI 2.7-12.9), p<0.0001. This remained significant also after adjustment for all conventional risk factors (HR 4.0, 95% CI: 1.73-9.11, p=0.001).

Table 4 - Baseline characteristics for study subjects with and without silent myocardial ischemia

	Silent Ischemia ***N*****=75 (11.1%)**	**No Silent ischemia** ***N*****=601 (89.9%)**	**P-value**
Age, y (mean ± SD)	66.9±6.6	64.2±6.8	0.001
Women, *n* (%)	39 (52%)	242 (40.3%)	0.052
Diabetes mellitus, *n* (%)	6 (8.0%)	69 (11.8)	0.37
Current smoker, *n* (%)	38 (50.7%)	274 (45.6%)	0.41
Systolic Blood pressure, mmHg (mean ± SD)	166.1±2	155.1±23.4	0.001
BMI, kg/m^2 (mean ± SD)	25.4±4.4	26.3±4.2	0.004
Cholesterol, mmol/l (mean ± SD)	6.1 ±1.0	6.0 ±1.1	0.74
Aspirin use, *n* (%)	13 (17.3%)	90 (15.0%)	0.59
β-blocker use, *n* (%)	6 (8.0%)	28 (4.7%)	0.21
Calcium-antagonist use, *n* (%)	6 (8.0%)	50 (8.3%)	0.92
ACE-inhibitor use, *n* (%)	5 (6.7%)	27 (4.5%)	0.40
Diuretic use, *n* (%)	14 (18.7%)	107 (17.8%)	0.85
NT pro-BNP, (pmol/l) Median (Q1-Q3)	9.15 (6.41-23.55)	6.32 (3.46-12.93) *n*=594	<0.0001
C-Reactive Protein, µg/m Median (Q1-Q3)	2.57 (1.07-4.27)	2.48 (1.17-4.59) *n*=597	0.57

4.3. Ventricular ectopic activity

Increased ventricular ectopic activity (> 30 VPC/h), was detected in 8.3% of the Copenhagen Holter Study population (56/678). Stroke rate was 3.6% in those with increased ventricular ectopic activity and 4.0 % in those without, Hazard ratio: 1.00, 95% CI: 0.24-4.22, p-value 0.99.

5. Patients with stroke and cardiac evaluation

Subjects with a recent stroke and sinus rhythm at admission time were evaluated by using electrocardiographic and cardiologic studies in a prospective consecutive study with focus on arrhythmias and coronary anatomy.

5.1. Methods and Study population

Recruitment

This study was originally planned as a single centre study but due to changes in health care organisation and restructuring of hospitals in the different regions of Denmark the study was also performed at a second centre. The study was initially performed from 1.02.2008 until October 2008 at the Copenhagen University Hospital of Amager and from December, first 2008 until September 2009 at the Copenhagen University Hospital of Bispebjerg.
All patients admitted to the acute medical care department of Amager Hospital and the acute neurological department of Bispebjerg Hospital with the diagnosis of stroke and/or TIA were screened (appendices).

Inclusion criteria

- Age >18 years.
- Admitted with neurological symptoms suspected of stroke (from 3 hours to 60 days after onset of symptoms), and verification of the diagnosis either by CT- or MRI scan.
- Sinus rhythm at admission.
- Ability to give informed consents either personally or by next of kin.

Exclusion criteria

- Paroxysmal or persistent atrial fibrillation.
- A history of previous severe stroke with modified Ranking Score (mRS) >3.
- A history of significant heart disease: previous MI, congestive heart disease, valvular heart disease.
- Elevation of Creatinin > 130 μmol/ml
- Cancer or severe other illness (expected of maximum six months of life)
- Pregnancy or breast feeding
- Dementia
- Allergy or hypersensitivity to contrast agents
- Uncertainty about diagnosis
- Subarachnoid or cerebral haemorrhage

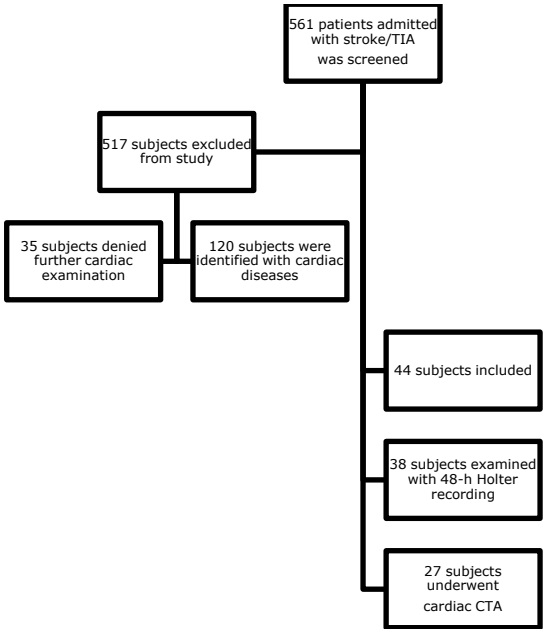
After inclusion all patients underwent a brief physical examination and blood tests. This was followed by a 48-hour Holter recording and a transthoraic echocardiography. Cardiac CTA was performed within two months after admission with ischemic stroke/TIA. The cardiac CT scanning was performed either at the radiological department of the University Hospital of Amager or the University Hospital of Copenhagen, Rigshospitalet.

Blood samples were taken at rest after 15 min and fasting blood samples stored as whole blood and serum for all patients. Holter recordings were initiated within few days. Trained personnel at the Holter laboratory, Copenhagen University Hospital of Hvidovre, analyzed flashcards containing data.

The echocardiographic analyses were done according to current guidelines[99;100]. Further details of study procedures are presented in appendices.

Bispebjerg Hospital had also centre function for treatment with recombinant tissue plasminogen activator (rt-PA) every second day. Patients with clear neurological deficits within four hours of index event and without doubt of diagnosis of ischemic stroke receiving a CT scanning of the brain uncovering no cerebral hemorrhagic and when blood pressure was appropriate were treated with rt-PA. Figure 6 demonstrates flow diagram of recruitment. Subjects (n=517) who were excluded from the study included also the 6.8 % subjects (n=35) who denied further cardiac examinations. The patients admitted and suspected for having a stroke or TIA, 295 subjects had been confirmed with the diagnosis of either stroke or TIA. Almost 12 percent denied being included in the study. More than 40 % were identified with cardiovascular diseases (n=120) including atrial fibrillation.

Figure 6 - Flow diagram of study population



Ethics

When eligible according to all inclusion and exclusion criteria, the patient and relatives were informed orally and in writing about study design and follow-up. Before inclusion all participants gave written informed consent. This study was approved by the regional ethical committee of Copenhagen (Region Hovedstaden, jr.-nr.H-C-2007-0074) and Datatilsynet (J.nr. 2007-41-0982).

5.2. Results

In total 561 subjects were screened and 44 persons were included in the study (Figure 7). From this population additional five patients did not complete all examinations: two because of deteriorating in clinical status, two because of logistical difficulties, and one died before completion of the study protocol. Thirty-eight subjects underwent 48-h Holter recordings. Three of whom were excluded because of poor recording quality. Only 27 subjects had performed cardiac CTA.

Characteristics of the study population are given in Table 5. The mean age was 62.8± years. Forty percent were current smokers and 17.1% had diabetes mellitus. ESVEA in patients with stroke: In 35 patients with a stroke were found 9 patients (25.7%) with ESVEA. Compared with subjects without stroke from the Copenhagen Holter study only 14.6% had ESVEA. But ESVEA was only borderline associated with stroke, p=0.07 (by using $\chi 2$ test). In a logistic regression model with ESVEA as dependent variable and systolic blood pressure, age and gender as independent variables, the association of ESVEA and stroke remained at the same borderline level (p= 0.066). In a forward selection model with the same variables ESVEA remained significantly associated with stroke in the model p=0.0414, and when taken in the model together with age (p<0.0001) and gender (p=0.004).

Table 5 - Baseline characteristics

Baseline Variables	***Subjects with successful Holter recording N=35***
Age, y	62.8+8
Female sex, n (%)	12 (34.3)
Current smoking, n (%)	14 (40.0)
Diabetes mellitus, n (%)	6 (17.1)
Total Cholesterol, mmol/l	5.02±1.09 N=28
Triglyceride, (mmol/l)	1.55±0.84 N=28
Glucose, (mmol/l)	7.3±2.76 N=25
Aspirin use, n (%)	9 (26.5) N=34
Statin use, n (%)	9 (26.5) N=34
Betablocker use, n (%)	1 (0.03) N=34
SBP, mmHg	168±26.9
DBP, mmHg	91.5±14.6

Cardiac CT-results

From all included study subject, 27 had a CTA done. One patient had image quality unsuitable for analysis of precise calcium quantity of the coronary arteries. All study subjects were identified with either plaques or stenosis of varying degrees. None were free of CAD. Sixty-three percent (17/27) of the population had coronary atherosclerosis without significant stenosis (> 50% narrowing of the lumen). Significant stenosis was found in (9/27) 33.3% of study subjects. Four subjects were diagnosed with one-vessel disease and four with two-vessel disease (14.8%). One subject was identified having three-vessel disease (5.9%). Table 6 illustrates prevalence of coronary atherosclerosis and vessel disease. No study subjects were identified with left main stem (LMS) stenosis. All study subjects had varying degrees of increased Agatston score. Plaque classification[101] were not assessed.

Table 6 - Prevalence of CAD and coronary artery calcium score in study subjects

	ALL, n=27
Coronary atherosclerosis without significant stenosis, n (%)	17 (63.0)
1 vessel disease, n (%)	4 (14.8)
2 vessel disease, n (%)	4 (14.8)
3 vessel disease, n (%)	1 (5.9)
Agatston score <400, n (%) [all=26]	18 (69.2)
Agatston score >400, n (%)	8 (29.6)

Agatston score was lower for women compared to men (Table 7), and 29.6 % (8/27) of the subjects had Agatston scoring above 400, indicating extensive evidence of CAD (Table 6). Subjects diagnosed with Agatston score >400 were all men.

Table 7 - Gender-divided Agatston score.

Gender	**Total score**	**Median of total score**	**Range**
Women	681	340,5	5 – 291
Men	8870	4435	0 - 1665

6. Discussion

6.1. Paper 1

ESVEA was associated with mortality or stroke after adjustment for other risk factors. An association with atrial fibrillation was also demonstrated with a 2.7-fold increased rate of atrial fibrillation in the follow-up period. For every increase of 10 SVEC per hour the risk of atrial fibrillation increased by 50% and the risk of the primary endpoint of death or stroke increased by 27%.

Study subjects were apparently healthy at enrollment, so ESVEA existed long before the event of stroke. Whereas it is known that atrial fibrillation is a risk factor for stroke, this adds to our knowledge of supraventricular arrhythmias being associated with stroke. So far no other population-based study estimated the risk of atrial fibrillation and mortality in relation to SVEC.

No accepted cut-off points of frequencies and runs of SVEC were known to be pathologic prior to this study. In general the risk of disease increases for any deciles increase in SVEC or runs. However, SVEC are very common and they are seen with different frequencies in almost all subjects. In this study it was supposed that SVEC should be excessive to increase the risk of disease significantly. Hence the top 10% (>30 SVEC /h) was decided to be used as cut-off point and therefore suggested as the top 10% of the incidence might be pathologic in this apparently healthy population (SVEC>30/h). However, separations into more groups and using a trend test instead showed with similar results.

In an association study, a high cut-off point usually leads to a significant association. But such a kind of cut off point turns to be useless due to low sensitivity. Nevertheless we consider the top ten percentile to be relevant. If this was to result in a prophylactic treatment, an investigation that identified 10% of the population and captured 10/27= 37% of the events would most likely be considered relevant. Further studies are needed to confirm our suggestion and results.

The origin and mechanism of increased SVEC are uncertain. Possibly increased SVEC might be a marker of a substrate (genetic or acquired) or another condition (HTN or pulmonary vein irritability for example) that is associated with atrial fibrillation or act as forerunners of atrial fibrillation. It may be an early manifestation of hypertension-related or another underlying structural heart disease that elevates filling pressures. Chronic hypertension can cause organ damage leading to diastolic dysfunction, enlargement of the left atrium that can potentially lead to increased atrial wall stress, increased SVEC and eventually atrial fibrillation.

The most likely mechanism is that increased SVEC is a harbinger of atrial fibrillation, but this remains to be demonstrated by serial Holter examinations (or with the insertion of a loop recorder). Whether increased SVEC and atrial fibrillation are epiphenomena of other heart diseases or conditions that also increase the risk estimates of death and stroke cannot be excluded. Paroxysmal atrial fibrillation (PAF) may also contribute to the incidence of stroke, but at present no studies have estimated the prevalence of strokes derived by PAF. Our results may indicate an indirect estimate of this risk, for the reason that subjects who have excessive supraventricular activity are at increased risk of PAF. Nevertheless SVEC is associated with death or stroke. This may still depend on development of clinically silent atrial fibrillation or an association with other structural heart disease.

6.2. Paper 2

Nighttime heart rate variability (HRV) was strongly associated with stroke in this study, whereas 24-h SDNN and MeanNN were associated with all-cause mortality, but not stroke. The observed increased risk associated with low nighttime HRV seems to be beyond conventional risk factors. Since reduced HRV has been demonstrated to be associated with many risk factors for atherosclerosis, including inflammatory markers[91] this may be a marker of other unmeasured confounders. No other studies have examined the prognostic value of reduced heart rate variability in stroke risk estimation.

The underlying mechanism between decreased nighttime HRV and increased stroke are not resolved, but several m nisms may be involved. Hypertension is most likely one of them. Some studies have found a strong correlation between HRV and blood pressure49. Reduced HRV, elevation in the heart rate and cardiac output, as well as increased levels of norepinephrine, characterize a hyperkinetic state that may delineate a transition state between borderline hypertension and high-resistant hypertension49. Other complications that are unrelated to blood pressure are increased hematocrit, tachycardia, obesity and insulin resistance49echa. Sympathetic activity maybe stimulates the enlargement of the left ventricle, thereby causing ventricular hypertrophy and stiffness of the arteries. This may trigger increasing vascular resistance[49].

Reduced parasympathetic tone may enhance hypercoagulation or increase blood viscosity, possibly triggering episodes of bradychardia and inducing arrhythmias.ref Variation in the heart rate has an impact on hypertension, atherosclerosis and cardiovascular morbidity and mortality. Impairment of the autonomic nervous system and the consequent sinus node derangement may develop wall stress in the atrium. It has been suggested that this may induce episodes of PAF, and even predispose to thromboembolism. We did not find any association between HRV and admissions for atrial fibrillation. Even so, admissions for atrial fibrillation most likely only compose a small part of the total burden of atrial fibrillation.

6.3. Silent myocardial ischemia

Our results showed an association between silent myocardial ischemia and stroke. The risk of stroke was increased by three-fold when having silent myocardial ischemia. Subjects with SMI had significantly increased rate of stroke. Subjects with SMI carry many risk factors for atherosclerosis that may also predispose for stroke. On the other hand SMI may represent a condition with advanced atherosclerosis[102] and thus increased possibility and risk of stroke. Our results are in good agreement with few reported clinical studies in this field; Kurl et al showed increased risk of stroke in subjects with SMI detected by exercise testing[58]. Silent myocardial ischemia might contribute to arrhythmias by weakening the myocardium. Some studies indicate an association of SMI and atherosclerosis and arrhythmias[103;104].

6.4. Cardiac CTA

This study demonstrated the burden of coronary atherosclerosis in stroke patients admitted with sinus rhythm. The prevalence is estimated to nearly 100 %. All study subjects had coronary atherosclerosis in different degree and one third had significant CAD. Differences in calcium score are seen between women and men. This is in good agreement with findings in other studies[105]. Physicians should have in mind that patients admitted with stroke might be referred for cardiac examinations. Preventive precautions may be indicated. Prevention may include lifestyle changes, smoking habits and control of blood pressure, cholesterol and diabetes. In addition to antithrombotic treatment following the stroke event, other medical treatment may consist of statins.

It is known from previous studies that reduced HRV is associated with risk factors for atherosclerosis. High calcium score is correlated with nighttime HRV but not with daytime HRV in this study.

The findings in the scanning indicate higher prevalence of CAD than findings by autopsies and coronary angiography. Cardiac CTA is possibly more sensitive and reliable than autopsies and coronary angiography. But this needs to be confirmed by other studies.

7. Conclusions

A 24-hour Holter recording provides additional prognostic information on the risk of stroke. Patients with stroke have a high risk of silent heart disease. This study revealed the extent of prevalence of unknown arrhythmias and ischemic heart disease. In particular, it was demonstrated:

- An association between excessive supraventricular ectopic complexes and stroke and atrial fibrillation.
- Nighttime HRV is strongly associated with stroke whereas 24-h HRV is associated with all-cause mortality. Both variables are associated with the combined endpoint of stroke or all-cause mortality.
- Silent Myocardial Ischemia as evaluated by Holter monitoring is associated with increased risk of stroke beyond conventional risk factors.
- ESVEA is more present in patients admitted with acute stroke or TIA particularly after correction with age and gender.
- In this study the extent of excessive coronary atherosclerosis was demonstrated and more than one third had significant stenoses in coronary arteries.
- This study confirmed that subjects admitted with stroke/TIA might be examined in more detail for ischemic heart disease and that ischemic stroke probably is more evident than previously thought.

8. Limitations and methodological considerations

The study population is exclusively middle-aged and elderly white and of Danish descent. No ethnic diversity is present and thus not reflecting ethnic pattern in the Danish general population or in other countries. So the data in this thesis should be used with caution on other ethnic groups.

Several other types of bias might be present in longitudinal surveys such as the CHS. A cohort of subjects is followed and outcomes were observed over time. Selection bias cannot be excluded since not all eligible subjects were able to or willing to participate.

However, loss of follow-up or recall bias was not present in this study. A loss to follow-up may not exist because of the national Danish Patient Registry was trawled at follow-up time by using the unique number of national Danish Civil Registration System for each citizen in Denmark. Surveillance bias cannot either be excluded. This type of bias might be eliminated or diminished by investigating all study subjects identical. Long-term studies may carry some troubles with changes of life habits over time. Demographic, social status and health related differences cannot be excluded (i.e. volunteers bias). Non-responders usually have more disease[96]. This generally applies to all studies where subjects are being invited by letter to participation. The limits of non-responding at follow-up time neither exist in this study. Discharge records of diagnosis of interest were reviewed in case of any doubts, but this does not rule out misclassifications.

With a view to avoid bias by only using one type of CT scanner (64-multislice Toshiba CT scanner) all image acquisitions were performed at AMH and Rigshospitalet, the Copenhagen University Hospital.

Endpoints and limitations

The numbers of cases with atrial fibrillation are small and might be underestimated due to lack of reporting and admissions. We have only been able to evaluate admissions for atrial fibrillation and many cases of paroxysmal atrial fibrillation may have passed undiscovered. It is possible that atrial fibrillation was treated as an outpatient so that there was no hospital admission and no discharge letters. Misclassification may also have been present. With respect to classification of atrial fibrillation (paroxysmal, persistent, or permanent), it was not classified into subtypes since this information was not always available. Minor or major influence on risk estimate cannot be excluded either because of the small number of endpoints. This applies especially when no association was found.

The small number of events limits both the power to detect associations and the ability to control completely for all potential confounders in the final multivariable models. We do not establish a cause-relationship, as our findings are associations. Thus, we are not able to conclude that ESVEA is more than a bystander in the development of stroke.

The primary endpoint was defined based on combined endpoint of all-cause mortality or first event of stroke in both papers. But the number of first event of stroke was only 27, and majority of the primary endpoints were all-cause mortalities (n=78). Therefore, the resulted association shown in the paper may represent the association of ESVEA with all-cause mortality rather than stroke. To cast light on this issue, further analysis was made, whereby association of SVEC and stroke was found at higher SVEC frequencies.

Cut-off points

The top ten percentile was decided as cut-off point regarding the study of ESVEA. No accepted cut-off point exists. In an association study, a high cut-off point usually leads to a significant association. But such kind of cut-off point turns to be useless due to low sensitivity. Nevertheless we consider the top ten percentile to be relevant. If this were to result in a prophylactic treatment an investigation that identified 10% of the population and captured 10/27= 37% of the events would most likely be considered relevant. Further studies are needed to confirm this suggestion and results. Runs of SVEC were classified as “runs” and no distinction was made between short bursts of atrial fibrillation or ectopic atrial tachycardia.

Other problems

With regard to the stroke study for the most elderly patients denied participation in further cardiac examinations. The more paralytic or severe neurologic deficits the more patients refuse being part of further studies. Many patient with severe acute stroke event refused further clinical investigation under admissions (n=35). Other patients with poor general status were not able to cooperate to cardiac examinations. Immediately after the study was implemented the nursing carried out a strike through several months in spring 2008, where the radiological department suspended the project.

In the late of the year 2009 the inclusion of patients with stroke were ended at AMH due to revisions in health care organisation and restructuring of hospitals in the different regions of Denmark the study was also launched at BBH. This posed further difficulties for the study and posed some logistical limits for study subject recruitment.

9. Clinical implications and future studies

Supraventricular ectopic complexes are quite common and about 10-15% of the middle-aged and elderly subjects with no apparent heart disease may have excessive supraventricular ectopic activity (ESVEA). This thesis shows that ESVEA in these subjects increases the risk of death or stroke, and atrial fibrillation, beyond conventional risk factor for atrial fibrillation.

It is currently not apparent how to treat these patients with ESVEA in order to reduce the associated risk of atrial fibrillation, death or stroke. Appropriate risk factor modification may be effective and in patients with hypertension antihypertensive regimens including blockade of renin-angiotensin system may reduce the incidence of atrial fibrillation[106]. Drugs aiming to maintain sinus rhythm in atrial fibrillation could also be tested in the patients with ESVEA. But this requires a future study. It is unknown if treatment of ESVEA can reduce future events.

Nighttime HRV may be used in relation to clinical perspective as an independent risk marker in risk stratification. Otherwise the predictive value is uncertain and interpretations have to be done with caution. The prognostic value of nighttime HRV has to be further investigated. It might be incorporated in risk score and a positive value could be additive to well-known risk scores. Patients with reduced nighttime HRV may be the targets for medication in order to avoid stroke in future trials. In case of heart rate variability derangement beta-blockage could be an opportunity but anticholinergic agents may be better when vagus overactivity is present49. Presently, nighttime HRV is not recommendable to be implemented in clinical use. It might be that nighhttime HRV could be part of some kind of scoring system. One could imagine that patients at high risk could be included in randomized interventional studies in view of nighttime HRV is a useful marker in risk stratification.

It could be that Holter positive (i.e. the presence of SMI, ESVEA or reduced HRV) findings should cause initiating of a randomized treatment study. However, the size of such a study will be large (inclusion of several thousand patients), but many new antithrombotic drugs could be relevant to test. In this study we provided a possible existence of new and potentially treatable risk markers, which could pave new measures to prevent stroke and stroke related disabilities.

Long-term Holter monitoring is recommended after stroke index with sinus rhythm in view of detection of paroxysmal atrial fibriallation[32;107]. Yet guidelines do not recommend long-term ECG recording in detection of paroxysmal atrial fibrillation routinely.

Physicians regularly see patients with ESVEA and reduced HRV during check-up examinations or other contexts. The comprehension that such supraventricular activity and reduced heart rate variability leads to a high risk may urge physicians to intensify risk modification, treat co morbidities more aggressively and perhaps increase patient compliance. Closer check up of these subjects is likely to identify subjects with appropriate preventive treatment as well as anticoagulant therapy. An interesting approach would be to search for subclinical ESVEA and atrial fibrillation in patients with other risk factors for stroke (hypertension, old age, diabetes).

From autopsies it is revealed a high prevalence of CAD in patients suffered from stroke, but our study indicates a higher occurrence. Coronary vessel disease were estimated to one third in patients with stroke/TIA indicating that physicians might have in mind that patients admitted with stroke/TIA and without known cardiac disease could suffer of coronary vessel disease and therefore be disposed to cardiac disease. This patient group does have the worst prognosis. Preventive treatment may prevent future stroke events and later the socioeconomic burden may be diminished.

Nevertheless there is a need of more investigation in this field. Further investigations in this field are important due to the socioeconomic burden of this patient group with ischemic stroke. Interventional studies in order to prevent later stroke or death in such patients are welcome.

It seems that incidence is more widespread than thought and primary prevention and treatment in early stage may prevent index stroke. A routine Holter examination can select persons at risk of stroke, where antithrombotic therapy should be considered.

10. Summary

This Ph.D thesis is based on 2 manuscripts and a prospective work of smaller size. The work was performed at medical/cardiological departments at both the Copenhagen University Hospital of Amager and Bispebjerg and the neurological department at Bispebjerg Hospital.

The first study aimed to find new and reliable risk markers for the development of stroke in a healthy population. The second study evaluated in which extent these risk factors (and other risk factors of interest) are prevalent in subjects with recent stroke. The intention of the last study was to examine patient admitted with a stroke, in order to determine the prevalence of silent arrhythmias and/or coronary artery disease.

Background

Stroke is worldwide one of the major causes of disability and death. In Denmark (5.5 mill. inhabitants) stroke is affecting more than 15,000 people annually, and the incidence is steadily increasing. After a stroke mortality is increased markedly. Many patients with stroke do also have cardiac diseases. Thirty percent of all stroke cases are believed to be of cardioembolic origin and atrial fibrillation accounts for half of these cardioembolic strokes. We examined whether miscellaneous Holter variables correlate with risk of stroke, death and atrial fibrillation in subjects without previous stroke or heart disease. Significant CAD is frequently seen in patients with stroke without diagnosed cardiac diseases.

Hypotheses

1. Miscellaneous Holter variables are independent risk markers for stroke.
2. Significant coronary disease is frequent in patients with stroke without recognized cardiac disease.

Methods

Study 1+2: The population-based cohort of Copenhagen Holter Study initiated in 1998-2000 and consisting of 678 healthy men and women, aging between 55 and 75 years, with no history of cardiovascular disease, atrial fibrillation, or stroke were evaluated. Various laboratory tests were conducted upon fasting patients and followed by 48-hour ECG monitoring. Median follow-up was 6.3 years. The results of a follow-up of the Copenhagen Holter study were used to examine whether some Holter variables can be used in risk stratification to avoid later stroke risk.

Study 3: The cohort of patient with acute stroke and sinus rhythm (n=44) were examined with 48-h ECG monitoring, fasting laboratory, echocardiography and referred to cardiac CTA. Twenty-seven subjects were examined with Cardiac CTA, a noninvasive tool to visualize coronary arteries and extent of calcification and atheromatosis. The presence of excessive atheromatosis and significant coronary stenosis (>50 % narrowing of lumen) in patients admitted with stroke/TIA without diagnosed cardiac diseases were revealed in this study.

Results

Paper I. Ninety-nine subjects (14.6%) had ESVEA. The risk of all-cause mortality or a stroke was significantly higher in subjects with ESVEA compared to those without ESVEA after adjustment for conventional risk factors. ESVEA was also associated with admissions for atrial fibrillation. SVEC, as a continuous variable, was also associated with stroke or death and admissions for atrial fibrillation.

Paper II. The risk of stroke was significantly associated with nighttime HRV in univariate and multivariate analysis per 10 ms reduction of HRV. The risk of stroke was associated with an increase by 33% after adjustment for relevant risk factors. Eighty-one percent of all strokes (21/26) occurred in subjects with low nighttime HRV (<38 ms), while in subjects with HRV >38 ms only 5 cases of stroke were seen (p=0.0016).

Study 3. Significant stenosis occurred in more than one third of study subjects admitted with stroke.

Conclusions

ESVEA in apparently healthy is associated with development of atrial fibrillation and is associated with a poor prognosis in term of death or stroke.

Nighttime HRV is a strong marker for development of stroke in apparently healthy subjects.

Cardiac CTA revealed a higher prevalence of atherosclerosis in patients with stroke than previously thought.

11. Dansk resumé (Danish summary)

Nærværende ph.d. afhandling er baseret på to manuskripter og et mindre prospektivt studie. Arbejdet er udført under ansættelser ved medicinsk/kardiologisk afdeling på såvel Amager Hospital og Bisbebjerg Hospital samt neurologisk afdeling ved BBH.

I det første studie forsøges at finde nye og stærke risikomarkører for udvikling af apopleksi i en sund population. I det andet studie evalueres i hvilket omfang disse risikofaktorer (og andre risikofaktorer af interesse) forekommer hos personer med nylig apopleksi. Hensigten med det sidste studie var at undersøge patienter indlagt med apopleksi med henblik på prævalensen af stumme supraventrikulære arytmier og/eller CAD.

Baggrund

Apopleksi er verden over en af de vigtigste årsager til invaliditet og død. I Danmark (5,5 mio. indbyggere) forekommer apopleksi hos mere end 15.000 mennesker årligt, og forekomsten er støt stigende. Mortaliteten er markant øget efter apopleksi. Mange patienter med apopleksi har også hjertesygdomme. Af alle tilfælde med apopleksi menes 30 % at være cardioembolisk oprindelse og atrial fibrillation er årsag til for halvdelen af cardioembolisk apopleksi. Vi undersøgte hvorvidt diverse Holter variable korrelerer med risikoen for apopleksi, mortalitet og atrial fibrillation hos personer uden foregående apopleksi eller hjertesygdomme. Betydelig CAD ses ofte hos patienter med apopleksi uden kendt kardiel sygdom.

Hypotese

1. Diverse Holter variable er uafhængige risikomarkør for apopleksi.
2. Signifikant koronar sygdom er hyppigt hos patienter med apopleksi men uden erkendt hjertesygdom.

Metoder

Studie 1 og 2: "Copenhagen Holter Study", er et epidemiologisk studie, indledt i 1998-2000, bestående af 678 raske mænd og kvinder mellem 55 og 75 år uden tidligere kardiovaskulær sygdom, atrieflimren, eller apopleksi, som blev rekrutteret. Alle inkluderede fik taget blodprøver (fastende) og en klinisk undersøgelse samt 48-timers EKG-monitorering. Den median follow-up tid var 6,3 år. Resultaterne af opfølgningen anvendtes til hvorvidt adskillige Holter variable kan anvendes i risikostratificering med henblik på senere hen at undgå udvikling af apopleksi.

Studie 3: Kohorten af patienter med akut apopleksi og sinusrytme (n = 44) undersøgtes med 48 timers Holter optagelse, faste blodprøver, ekkokardiografi og derpå henvist til hjerte-CT. Syvogtyve personer blev undersøgt med hjerte-CT, et noninvasive metode til at visualisere koronar kar og omfanget af calcificering samt athe romatose. Forekomsten af udtalt atheromatose og betydelig koronar stenose (> 50 % lumen forsnævring) hos patienter med apo pleksi/TCI uden kendt kardiel sygdomme blev afsløret i dette studie.

Resultater

Studie 1: Nioghalvfems personer (14,6 %) havde ESVEA. Risikoen for mortalitet eller apopleksi var signifikant højere hos personer med ESVEA i forhold til dem uden ESVEA efter justering for konventionelle risikofaktorer. ESVEA var også associeret med indlæggelser for atrial fibrillation. SVEC, som kontinuerlig variabel, var associeret med apopleksi eller død og indlæggelser med atrial fibrillation.

Studie 2: Risikoen for apopleksi var signifikant associeret med natlig HRV i såvel univariate og multivariate analyse pr. 10 ms reduktion af HRV. Risikoen for apopleksi var associeret med en stigning på 33 % efter justering for relevante risikofaktorer. 80 % af alle apopleksier (21/26) forekom hos personer med lav natlig HRV ($\leq$38 ms), mens kun 5 tilfælde af apopleksi blev set hos personer med natlig HRV (>38 ms), (p = 0,0016).

Studie 3: Betydelig koronar stenose forekom hos mere end en tredjedel af forsøgspersonerne indlagt med apopleksi.

Konklusioner

ESVEA er forbundet med udvikling af atrieflimren og ringe prognose med hensyn til mortalitet eller apopleksi.

Natlig HRV er en stærk markør for udvikling af apopleksi hos tilsyneladende raske personer.

Studiet viser en højere forekomst af CAD hos patienter med apopleksi end hidtil antaget.

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Excessive Supraventricular Ectopic Activity and Increased Risk of Atrial Fibrillation and Stroke

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Background—Prediction of stroke and atrial fibrillation in healthy individuals is challenging. We examined whether excessive supraventricular ectopic activity (ESVEA) correlates with risk of stroke, death, and atrial fibrillation in subjects without previous stroke or heart disease.

Methods and Results—The population-based cohort of the Copenhagen Holter Study, consisting of 678 healthy men and women aged between 55 and 75 years with no history of cardiovascular disease, atrial fibrillation, or stroke, was evaluated. All had fasting laboratory tests and 48-hour ambulatory ECG monitoring. ESVEA was defined as ≥30 supraventricular ectopic complexes (SVEC) per hour or as any episodes with runs of ≥20 SVEC. The primary end point was stroke or death, and the secondary end points were total mortality, stroke, and admissions for atrial fibrillation. Median follow-up was 6.3 years. Seventy subjects had SVEC ≥30/h, and 42 had runs of SVEC with a length of ≥20 SVEC. Together, 99 subjects (14.6%) had ESVEA. The risk of primary end point (death or stroke) was significantly higher in subjects with ESVEA compared with those without ESVEA after adjustment for conventional risk factors (hazard ratio=1.64; 95% confidence interval, 1.03 to 2.60; P=0.036). ESVEA was also associated with admissions for atrial fibrillation (hazard ratio=2.78; 95% confidence interval, 1.08 to 6.99; P=0.033) and stroke (hazard ratio=2.79; 95% confidence interval, 1.23 to 6.30; P=0.014). SVEC, as a continuous variable, was also associated with both the primary end point of stroke or death and admissions for atrial fibrillation.

Conclusions—ESVEA in apparently healthy subjects is associated with development of atrial fibrillation and is associated with a poor prognosis in term of death or stroke. **(*Circulation*. 2010;121:1904-1911.)**

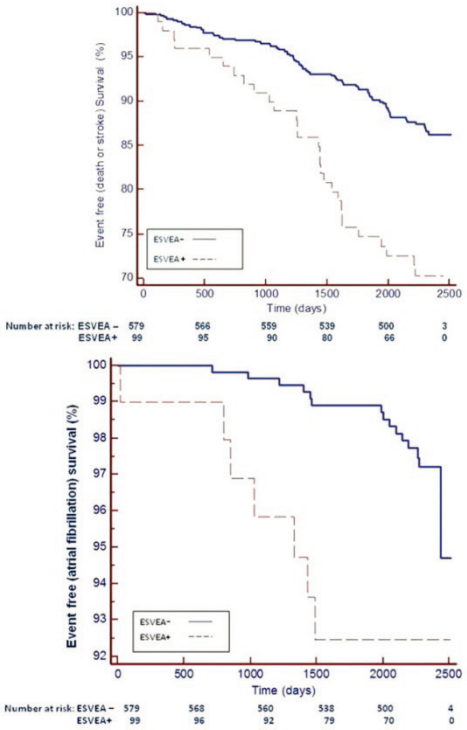
Key Words: arrhythmia ■ prognosis ■ risk factors ■ stroke

CLINICAL PERSPECTIVE

Supraventricular ectopic complexes are quite common, and ≈10% to 15% of middle-aged and elderly subjects with no apparent heart disease may have excessive supraventricular ectopic activity. This study shows that excessive supraventricular ectopic activity in these subjects increases the risk of death, stroke, and atrial fibrillation beyond conventional risk factors or the Framingham risk score for atrial fibrillation. Physicians frequently see patients with excessive supraventricular ectopic activity during routine examinations or in other contexts. The finding that such supraventricular activity leads to a high risk may urge physicians to intensify risk modification, treat comorbidities more aggressively, and perhaps increase patient compliance. Closer follow-up of these subjects is likely to identify subjects with asymptomatic atrial fibrillation and thus promote appropriate preventive treatment as well as anticoagulant therapy. Interventional studies to prevent atrial fibrillation and later stroke or death in such patients are welcome.

Correction

In the article by Binici et al, "Excessive Supraventricular Ectopic Activity and Increased Risk of Atrial Fibrillation and Stroke," which appeared in the May 4, 2010 issue of the journal (*Circulation*. 2010;121:1904–1911), there was an error in Figure 1. In panels A and B, the survival curves representing "ESVEA+" and "ESVEA−" were mislabeled. The corrected Figure appears below:



Decreased Nighttime Heart Rate Variability Is Associated With Increased Stroke Risk

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Background and Purpose—Prediction of stroke in healthy individuals is challenging and there is a diurnal variation of stroke onset. We hypothesized that heart rate variability with a focus on nighttime heart rate variability will predict the risk of stroke in apparently healthy middle-age and elderly subjects.

Methods—The population-based cohort of the Copenhagen Holter Study, consisting of 678 healthy subjects between age 55 and 75 years with no history of cardiovascular disease or stroke, was evaluated. All underwent 48-hour ambulatory electrocardiogram monitoring. The SD of normal-to-normal RR intervals (SDNN) was selected as the method of measuring heart rate variability. Nighttime SDNN was measured between 02:00 and 02:15 AM and could be evaluated in 653 subjects. Median follow-up was 76 months.

Results—Nighttime SDNN was lower in women than in men ($P=0.0008$), and in diabetics than nondiabetics ($P=0.03$). However, smoking, cholesterol, systolic blood pressure, and age were not associated with nighttime SDNN. The risk of stroke was significantly associated with nighttime SDNN in a univariate analysis (HR, 0.66; 95% CI, 0.50–0.88; $P=0.004$) and after adjustment for conventional risk factors (HR, 0.67; 95% CI, 0.51–0.89; $P=0.005$) per 10 ms increments of SDNN. Eighty-one percent of all strokes (21/26) occurred in 330 subjects with the lower half of nighttime SDNN (≤38 ms; HR, 4.31; 95% CI, 1.62–11.42; $P=0.003$).

Conclusions—Nocturnal heart rate variability is a strong marker for the development of stroke in apparently healthy subjects. The mechanism is unknown, but reduced parasympathetic activity may increase the risk of stroke by increasing the risk of arrhythmias. **(*Stroke*. 2011;42:3196-3201.)**

Key Words: nighttime heart rate variability ■ SDNN ■ atrial fibrillation ■ risk factors ■ stroke

Case Report Form: Study 3

When enrolled in the study following examinations were done:

Baseline information was obtained:

- Risk factors: familial predisposition, hyperlipidemia, hypertension, smoking habits, diabetes mellitus, claudicatio intermittens and physical activity level (se appendix A).
- Concomitant diseases
- Use of medication

Clinical examination:

- Weight, Height, waist/hip
- Blood pressure, pulse rate
- Clinical examination (St.p. et c.)
- Neurological classification (TOAST criteria), classification of the neurological deficits: SSS (Scandinavian Stroke Scale) and modified Rankin score (se appendix C and E).

Echocardiography:

- Left and right ventricle ejection fraction
- Measure of left atriums size and volume
- Valvular abnormity
- Measures of diastolic function

Holter recording:

48-hours continuous 12-lead ECG monitoring with recording of:

- Heart rate and heart rate variability (24-h, day-time, nighttime).
- Ventricular ectopic activity / arrhythmias.
- Supraventricular ectopic activity / arrhythmias.
- Heart rate variability (24-h, day-time, night-time): MeanNN, SDNN, SDANN, SDNNindex, PNN50, RMSD

Laboratory investigations:

- Na, K, Creatinin, Hgb, thrombocytes, Leucocytes, Fibrinogen, D-dimer, INR, ASAT, ALAT; bilirubin, CRP, Lipids (LDL; HDL; triglycerides)
- Plasminogen activator inhibitor (PAI)
- Troponin T
- NT-pro BNP

Radiology:

- CAT scanning of brain.
- CAT scanning of heart

CT scan protocol

As previously described by Kristensen et al the patients underwent a cardiac CTA[91;92].

Briefly, an intravenous bolus injection of X-ray contrast is administered and additionally ECG/blood pressure is monitored. An ECG-gated coronary CT-angiography was constructed due to well-defined guidelines (Toshiba Aquillion 64 multislice CT, Visipaque) – estimated duration of examination is 30 minutes. Contrast agent: Visipaque (General Electric Healthcare, Princeton, New Jersey, USA)

80-100 ml depending on weight and BMI, iodhexodol 320 mg/cm^3.

Image acquisition

Images were performed by using a Toshiba Aquillion 64 multislice CT system (Toshiba Medical Systems, Otawara, Japan). Images were obtained during a single breath-hold in inspiration. Before the examination all patients were giving according to guidelines and procedure orally beta blockage and sublingual Nitroglycerin.

Images were evaluated done at Vitrea (version 3.9, Vital Images, USA) workstation by to observers (KK, TK). Arteries were segmented according guidelines from American Heart Association.

Aspects of radiation hygiene

A conventional CT scanning of thorax involved the whole thorax and the effective dose is about 9 mSv. In a coronary CT-angiography only the half of the thorax is imaged and irradiated. Additionally reduction of the X-ray radiation exposures can be achieved by the use of new techniques and software. The effective dose is thus diminished to 6 mSv. In comparison a myocardial perfusion SPECT scanning irradiate with a dose of 10 mSv.

It's theoretically calculated that radiation dose rises the risk of radiation-induced cancer is 1 out of 3000, this corresponded to an increase in radiation induced risk of 25% for background population to 25.03% as result of the CT-scanning.

Sievert definition: The radiation is measured in sievert. To put all ionizing radiation on common ground with regard to potential for causing harm, a radiation-weighted dose called the equivalent doses is introduced. The sum of the weighted equivalent doses is called the effective dose. The term 'dose' refute to the committed effective dose, which is expressed in sieverts (Sv). 1 Sv = 1 Sievert = 100 rem = 1 J/Kg

(The Danish National Institute of Radiation Hygiene has done these calculations.)

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