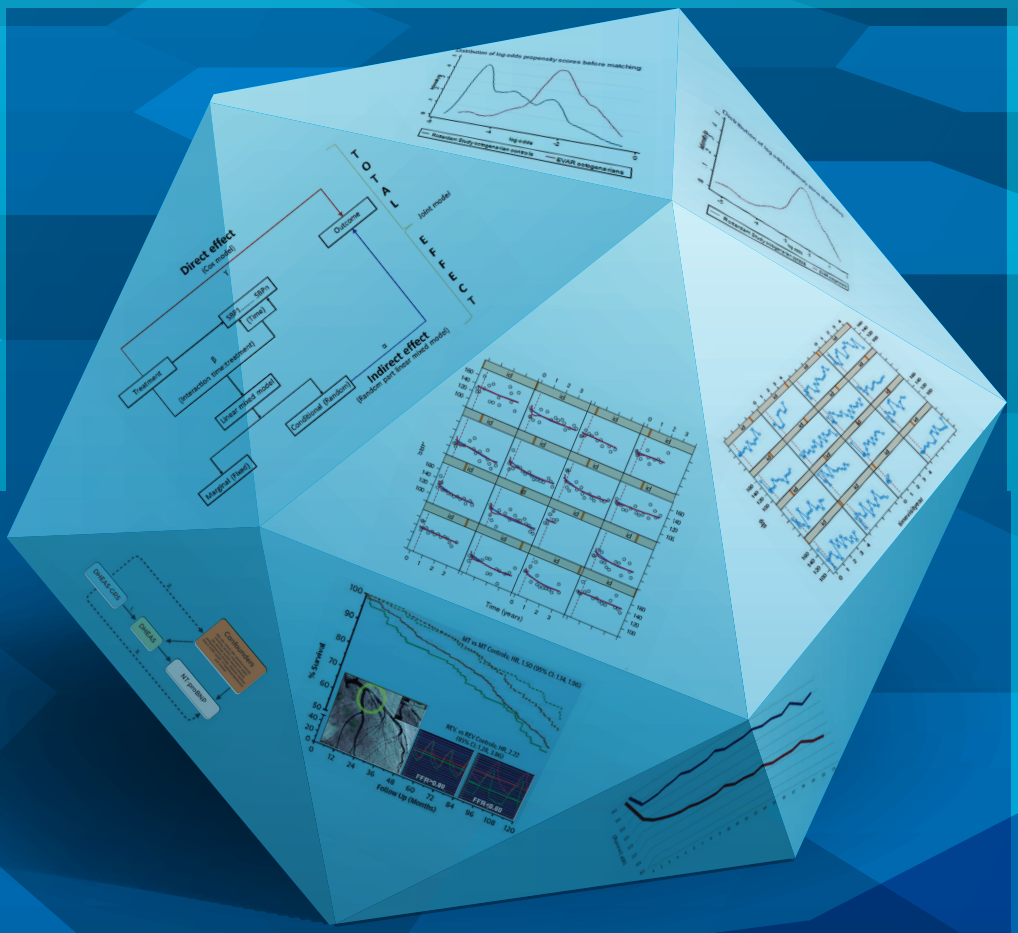
Methodological and Epidemiological Studies of Cardiovascular Structure and Function



Oscar Leonel Rueda Ochoa

Methodological and Epidemiological Studies of Cardiovascular Structure and Function

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Methodologische en Epidemiologische Studies van Cardiovasculaire Structuur en Functie

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Methodological and Epidemiological Studies of Cardiovascular Structure and Function

Cover: The Icosahedron is the fifth of the Plato`s solid shapes. For Plato`s association, it represents the element of water. Water is all about movements, flow and change. In the Metaphysical interpretation, Icosahedron is the center of creativity on both the physical and mental realms. All figures included into the Eicosahedron of the cover page are the main findings of this thesis.

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Ethics Approval

The Rotterdam Study has been approved by the medical ethics committee according to the `Wet Bevolkingsonderzoek: ERGO` (`population Screening Act: Rotterdam Study`) executed by the Ministry of Health, Welfare, and Sport of the Netherlands.

Participants of the Rotterdam Study (RS) provided written informed consent to participate in the study at enrollment and at each repeat examination, and to obtain clinical information from their treating physicians, separately. The latter includes permission to obtain information from the general practitioner, medical specialists, and pharmacists.

“Longitudinal data analysis in neonatal electrocardiogram” and “Impact of cumulative systolic blood pressure and serious adverse events on efficacy of intensive blood pressure treatment: a randomized clinical trial” were approved by the ethics committee of Universidad Industrial de Santander UIS, in Bucaramanga, Colombia.

Dedicated to Isaura Ochoa, Lyda Zoraya Rojas, Mila and all my past, present and future students from Universidad Industrial de Santander, UIS, in Bucaramanga, Colombia.

Paranymphs

Carolina Patricia Ochoa Rosales
Gertrudis Elizabeth Benz Inalaf

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This thesis is based on articles published in various scientific journals. Differences may exist in exact wording and abbreviations between the text in this thesis and the text of the published version of the articles due to editorial changes and linguistic differences. Permission to reproduce the individual chapters in this thesis was obtained from the publishers of the various scientific journals.

List of Abbreviations

BioLINCC: Biologic specimen and data repository information coordinating center.

BMI: Body mass index

BSA: Body surface area

CHD: Coronary heart disease

cJM: Cumulative Joint Model

CKD: Chronic Kidney disease

CVD: Cardiovascular disease.

DBP: Diastolic blood pressure

DM: Type 2 diabetes mellitus

HF: Heart failure

HFpEF: Heart failure with preserved ejection fraction

HFrEF: Heart failure with reduced ejection fraction

HR: Heart rate

LAD: Left atrial diameter

LMM: Linear mixed effect model.

LVDF: Left ventricular diastolic function

LVEF: Left ventricular ejection fraction

LVM: Left ventricular mass

pdDiag: diagonal covariance matrix.

SAEs: Serious adverse events

SBP: Systolic blood pressure

SPRINT: Systolic blood pressure intervention trial

CHAPTER 1

General Introduction

CHAPTER 1.1
Introduction

1.1

The development of health sciences research goes hand in hand with advances in the design of epidemiological studies as well as the mathematical tools for the analysis of biomedical information. Initially, medical research focused on the description of the findings observed in individual patients, with a detailed description of the signs and symptoms and the assignment of names to them. With growing understanding of epidemics, the need arose to keep a record of the observed cases, detailing their characteristics, which should be summarized for its interpretation, using simple mathematical measures such as frequency in percentages and measures of central tendency like the average and median. In this way, the initial interest of the individual case progressed to the study of groups of people who shared a common disease. With the pandemic of cardiovascular diseases, population-based studies were assembled, such as the Framingham Heart Study and the Rotterdam Study, following participants until the development of clinical events of interest. Also, progress was made from only the descriptive interest to the interest in unravelling the determinants associated with the ocurrence of diseases which implied the development of new methodological tools such as linear, logistic, binomial, Poisson regression and Cox proportional models, among others.

In recent years, a new interest has emerged to understand how, in turn, dynamic changes in clinical variables, measured repeatedly over time, could be associated with determinants and may, per se, be associated with the development of adverse events (Clinical outcomes). This new interest has been consolidated, thanks to the development of new statistical analysis tools such as the Cox proportional hazard model and its extensions, linear models for repeated measurements of continuous data such as linear marginal and mixed-effects model and generalized estimation equations (GEE), and joint models for longitudinal and to time-to-event data, among others. In turn, new statistical techniques, such as propensity score matching, have allowed improving the quality of the results obtained from observational studies by providing further tools to deal with bias and confounding. Advances in the area of genetic epidemiology such as the development of genetic risk scores and Mendelian randomization analysis, have narrowed the gap between association studies with those aiming to establish causality.

Challenges in the analysis of repeated measurements

Interest in the study of repeated measures has gained enormous importance in recent years due to the emergence of an increasingly growing number of Cohort studies with long follow-ups and multiple measures of both outcomes and covariates with potential to be clinical biomarkers. In these studies, the common assumption of independence of observations is not satisfied, making the use of classical statistical methods, such as student t-test and ANOVA, not applicable. It is necessary to implement methods that appropriately account for these correlations. In addition, in conditional models, the random effects given by the variability of the data within individuals must be considered. Also, linear and non-linear relations must be considered in the progression of the outcomes through time. In turn, the differences between groups (Marginal approach) and the differences within individuals (conditional approach) are aspects that should be explored in these models. In addition, given the greater mathematical complexity, problems of convergence in the models and greater computational requirements have increased.

Overall, despite great methodological and statistical advances, great challenges remain in the repeated measurements analysis as strict analytical assumptions should be satisfied and specific analytical procedures followed. Among the assumptions to consider are: linearity, homoscedasticity (constant variance), normal distribution of error terms and random effects, missingness at random (MAR), and the choice of the correct covariance matrix adjusted to the repeated outcomes. Failure to meet the special requirements can make studies with repeated measurements vulnerable to statistical errors and can lead to incorrect conclusions. In chapter 2.1, 2.2 and 2.3, we have applied repeated measurements analysis and we have assessed all statistical assumptions required to obtain results with high internal validation.

Joint Model analysis: A powerful statistical tool for combining the longitudinal and time-to-event data analysis

In recent years, Joint modeling (JM) analysis has emerged as a novel approach that evaluates the association of biomarkers measured repeatedly over time with time to clinical outcomes. In these models, the repeated measures of the biomarker are the outcome of a linear mixed model, with a fixed component and a random component. The fitted values of the longitudinal trajectory for each individual are used as covariates in a time-varying Cox proportional hazards model. The advantages of this type of model are multiple, compared to the independent analysis of the biomarker and the extended Cox models, including: i) the joint

modeling approach does not condition on the biomarker after randomization but rather treats it as an outcome, ii) it accounts for the correlations in the repeated biomarker measurements per patient and iii) it accounts for missing at random missing data in the longitudinal biomarker.

JM analysis allows to investigate the association of the repeated measurements of the biomarker with the clinical outcomes in different ways and helps to understand the mechanisms that could explain this association. Using this methodology, we can answer questions such as: Is organ damage produced by higher punctual values of the biomarker immediately before the clinical outcome (standard JM)? Or is it produced by biomarker cumulative effect (Cumulative JM)? Or is it produced by changes in the slope of the trajectories of the biomarker over time (Slope JM)? What about the biomarker variability as responsible of organ damage? (intra-individual and between groups).

Regarding Joint modelling analysis, a large number of different models have recently been proposed. Most considered jointly modelling linear mixed models with proportional hazard models, with correlation between multiple longitudinal outcomes accounted for through multivariate normally distributed random effects. So-called current value and random effects parameterizations are commonly used to link the models. Despite the developments, appropriate fast-processing softwares are still lacking, which has translated into limited uptake of such models by medical researchers. Additionally, although in an era of personalized medicine the value of multivariate joint modelling has been established, researchers are currently limited in their ability to fit these models routinely.

In this thesis, we made a secondary analysis of the SPRINT trial applying a cumulative JM approach (chapter 2.3), an advanced statistical method to include time-varying covariates. Here, a summary of the entire history of the longitudinal systolic blood pressure (SBP) measurements up to time t is included in the hazard model, $\lambda i(t)$. This is contrary to other association structures that relate the hazard function only to features of the longitudinal model at a fixed time point.

Propensity score matching to decrease bias in observational studies

The ideal method to evaluate the efficacy of an intervention is the randomized controlled clinical trial. However, on many occasions, due to ethical limitations, it is not possible to carry out these type of studies and it is necessary to use data collected in observational studies. Unfortunately, observational studies are limited

by the potential risk of bias and confounding, which limits their validity to evaluate the efficacy of an intervention. In recent years, new statistical tools, such as propensity score matching, have been developed which aim to increase validity by reducing biases. Propensity score matching is a method that is based on the choice of the best control group taking into account previously defined characteristics that may be associated with receiving or not receiving the intervention.

Using a logistic model for the dichotomous outcome of receiving or not receiving the intervention, all the variables that are considered to potentially influence this decision are accounted for. The predicted values of this model (propensity score) are obtained and, with these values, a match is made between the individuals with the closest scores, of the groups under study, which defines that they are similar in the characteristics under study. This method greatly reduces confounding by improving the comparability between the groups. However, latent variables are not taken into account, so their limitation persists in this area compared to clinical trials that balance groups by both measured and unmeasured variables (latent) at baseline. Compared to the selection of controls using the classic pairing only by age and sex, propensity score matching has shown to have a greater power to detect differences between the groups under comparison, in addition to having smaller biases.

However, propensity score matching have challenges including the adequate selction of covariates to include in the model and the choice of the best method to use for the statistical analysis since no coherent, rule-based decision matrix currently exists in the literature. This statistical method was applied in chapters 3A.1 and 3A.2.

Polygenic risk score and its contribution to disentangling biological pathways

Polygenic risk score (PRS) is currently the method most frequently used to predict the genetic risk of complex human diseases, based on findings obtained from genetic wide association studies (GWAS). PRSs are calculated by multiplying the number of risk alleles that a person has by the size of the effect of each variant, obtained from GWAS studies, and then adding each of these products to all risk loci.

PRSs can be used for studying the correlation between pairs of genotype-phenotype associations and to examine biological pathways for phenotypes that have been measured at sufficient scale to generate well-powered GWAS summary statistics.

However, they cannot be taken as evidence of causality mainly due to the fact that the large number of SNPs typically used in their calculation usually have highly pleiotropic influences.

At present, the clinical utility of genetic risk prediction is still limited. However, there is significant promise for future clinical applications as the ancestral diversity and sample sizes of GWAs studies increase. The future of genetic risk prediction is anticipated to benefit several areas of research and clinical practice as personalized medicine.

Mendelian Randomization, going from association studies to causality studies

Observational studies allow assessing scientific hypotheses. This type of studies evaluate the correlation between an exposure factor and a disease, generating hypothesis regarding its association. However, this association cannot be interpreted as a causal relationship, mainly due to unmeasured confounding (residual confounding) and reverse causation. More robust approaches are needed for assessing causal relationship using observational data. Mendelian randomization is one such an approach. It aims making inferences about causal effects based on observational data using genetic instrumental variables.

An instrumental variable is associated with the exposure under study, but not associated with any other confounding factor. Neither is it associated with the outcome directly. It is associated with the outcome only through the exposure of interest. In Mendelian randomization, genetic variants are used as instrumental variables and the main methodological challenge is to evaluate the assumptions of the instrumental variable and the possible violations of them. Among the possible violations to consider are the pleoitropy, canalization, linkage disequilibrium, effect modification, stratification and ascertainment effects.

CHAPTER 1.2

Aim, setting and outline of this thesis

This thesis focuses on application of novel epidemiological and methodological tools, aiming at contributing to answer research questions that involve dynamic changes in clinical variables, measured repeatedly over time, that are related to cardiac and vascular structure and function and their changes over time. Also, more recently used and advanced methods such as propensity score matching, polygenic risk scores and Mendelian randomization are used to evaluate causal inference in quasi-experimental assays and cardiovascular biomarker studies respectively. Finally, we evaluate the distribution of ascending and descending thoracic aortic diameter in the elderly population and their association with major adverse cardiovascular outcomes, using Cox proportional hazard and competing risk analysis.

This thesis aimed to answer the follwoing research questions, applying advanced statistical methods as:

I. Repeated measurements analysis

1. How are the changes in newborn electrocardiogram during the neonatal period? Which risk factors are associated with these changes? How do these results compare with the current European society consensus on neonatal electrocardiography?

2. What are the changes in left ventricular diastolic function parameters, measured by echocardiography, over time among men and women of the Rotterdam study? Which risk factors are associated with these changes? Are there gender differences in the trajectories and risk factors associated with left ventricular diastolic parameters?

II. Joint modelling analysis

3. How do repeated systolic blood pressure measurements, systolic blood pressure variability and serious adverse events affect the efficacy of intensive treatment of systolic blood pressure in the SPRINT trial?

III. Propensity score matching

4. How is the survival after endovascular aortic aneurysm repair (EVAR) in a cohort of octogenarians compared with octogenarians without abdominal aortic aneurysm from population-based the Rotterdam study cohort, selected using propensity score matching?

5. How is the survival after fractional flow reserve guide treatment in a cohort of coronary heart disease patients with intermediate coronary stenosis compared with a control group, from the population-based Rotterdam study cohort, matched using propensity score matching?

IV. Mendelian randomization analysis

6. Is there a causal relationship between dehydroepiandrostenedione sulfate (DHEAs) and N-terminal of type B natriuretic pro-peptide (NT-Pro-BNP)? Are there gender differences in this association?

V. Multiple linear regression, Cox proportional hazard analysis and competing risk analysis

7. What are the reference values for thoracic aortic diameters in a healthy population older than 55 years from the Rotterdam study? Which risk factors are associated with the thoracic aortic diameters? Are there gender differences in the distribution of the thoracic aortic diameters?

8. Are thoracic aortic diameters associated with major adverse cardiovascular outcomes? Are there gender differences in the associacion of thoracic aortic diameters with major cardiovasdcular outcomes?

VI. Polygenic risk score

9. Is a polygenic risk score for coronary artery disease associated with atherosclerosis in various vascular beds?

All papers of this thesis are organized in the next chapters:

Chapter 2 focuses on methodological studies of longitudinal repeated measurements. Three papers contribute to this chapter. The papers are based on repeated measurements of the variables that evaluate changes in hemodynamic and cardiac structure by electrocardiogram (chapter 2.1), functional changes by

echocardiogram (chapter 2.2), and changes over time in systolic blood pressure (chapter 2.3). Novel methods like marginal, mixed, GEE and cummulative joint models are used in these analysis. The aim of this chapter is to evaluate which factors are associated with changes in the parameteres under study over time (chapter 2.1 and 2.2) and to evaluate how changes in systolic blood pressure over time could be associated with cardiovascular outcomes affecting the efficacy of medical interventions (chapter 2.3). This chapter further includes two letters to the editor focusing on the discussions about the role that serious adverse events could play in the efficacy of intensive systolic blood pressure treatment and the validity of our secondary joint model analysis of the original SPRINT dataset (chapter 2.3.1 to 2.3.2).

Chapter 3 focuses on advanced methods for causal inference. This chapter includes three papers; two of them regarding the selection of adequate controls in quasi-experimental designs to evaluate the efficacy of medical interventions (chapter 3.A.1 and 3.A.2), and the third using a Mendelian Randomization approach to evaluate causality between two hormones relating to cardiac and vascular structure and function (Chapter 3B.1).

Chapter 4 is focused on epidemiological studies of aortic structural and function. This chapter includes three papers from the Rotterdam Study. In the first two papers (chapter 4.1, 4.2) we evaluate the distribution of ascending and descending thoracic aortic diameters in the elderly population. Also, the association of ascending and descending thoracic aortic diameters with major adverse cardiovascular outcomes; such as stroke, coronary heart disease, heart failure, cardiovascular and total mortality are evaluated. In the third paper (Chapter 4.3), the hypothesis that atherosclerosis is a unique global condition mediated by a common genetic profile is evaluated using a coronary artery disease genetic risk score based on 160 single nucleotide polymorphisms (SNPs) reported in most recent GWAS. The genetic risk score is tested for association with clinical and subclinical atherosclerosis outcomes in different vascular beds; including coronary artery calcification, extra- and intra-cranial carotid calcification, aortic arch calcification, pulse wave velocity, ankle-brachial index, incidence of ischemic stroke, incidence of coronary heart disease, incidence of cardiovascular death and prevalence of peripheral artery disease.

Finally, in chapter 5 a general discussion about the main findings and methodological considerations on the research described in this thesis is presented.

CHAPTER 2

Methodological studies of longitudinal repeated measurements

CHAPTER 2.1

Longitudinal data analysis in neonatal electrocardiogram

Accepted Fire Poster Oral presentation, European Society Cardiology Congress 2016.

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ABSTRACT

Background: Dynamic changes in neonatal electrocardiography patterns remain unclear. We aimed to evaluate the longitudinal changes in the electrocardiographic parameters in a cohort of healthy newborns during the neonatal period and to identify maternal and perinatal factors associated with these changes.

Methods: Prospective cohort study including 120 healthy neonates born in a general hospital who were the product of a normal full-term pregnancy. The ECG signals were taken on the 1st, 7th, and 30th days after birth using a digital device. All waves, segments, and intervals were measured. All readings were validated first visually and then by a pediatric cardiologist.

Results: Heart rate, amplitude P wave in DII, and amplitude R wave in V5 increased significantly in the ECGs from the 1st day to the 7th and the 30th day. Interval PR on the 7th day ECG decreased significantly. QTc interval in V3R, amplitude S wave in V1, and amplitude S wave in V5 decreased significantly on both the 7th and the 30th day ECGs. QRS duration in V5 and amplitude R in V1 decreased significantly on the 30th day. R/S ratio increased in V5 on the 7th and 30th day ECGs. Birth weight, age of the mother, number of gestations, and caesarean section were significantly associated with changes in ECGs neonatal parameters.

Conclusion: Within the first month of life, there are large dynamic changes in electrocardiographic parameters. Such dynamic changes could shed more light on pathological conditions such as congenital cardiopathies and sudden infant death.

Keywords: Neonatal Screening, Electrocardiogram, Repeated measurements, Linear mixed model, Risk factors, Cohort Study.

Introduction

During the neonatal period (first 30 days after birth), newborns experience large physiological challenges from changes in multiple systems but especially in their cardiorespiratory function. This is mainly due to changes in the distribution of fetal circulation including changes in pulmonary vascular resistance and closure of the ductus arteriosus and foramen ovale[1].

A close monitoring of these cardiovascular changes could be done using periodic electrocardiogram (ECG) assessments to evaluate heart rhythm, bundle branch blocks, atrioventricular blocks, and cardiac repolarization disorders (such as prolonged QT syndrome). However, thorough knowledge of normal and pathological diagnostic criteria in electrocardiography among neonates is lacking[2,3].

The current consensus regarding normal neonatal electrocardiogram[4] is based on two studies. The first done by Davignon et al. (published in 1979) and studied 2141 children (668 neonates)[5]. Results from this effort were hampered by low sampling rate and low bandwidth for recording the ECGs. Furthermore, the measurements were conducted at a single time point without repeated evaluations of the dynamic process. The second study is a more recent effort by Rijnbeek et al. (published in 2001)[6] which was conducted in a small sample of newborns older than 10 days (n = 44) with measurements taking place at a single time point as well. Updated information with a closer monitoring and longitudinal evaluation is fundamental to better characterize the dynamic cardiovascular adaptations in neonates.

Hence, we aimed to evaluate the electrocardiographic dynamic changes in a cohort of healthy newborns followed during the neonatal period while following the current recommendations for digital ECG registration and to identify the factors associated with these changes.

Methods

Study population and design

Our study was conducted at the University Hospital of Santander in Bucaramanga, Colombia. From the 869 consecutive births at the University Hospital of Santander, we selected the neonates who met the following criteria: (1) their mothers had attended prenatal visits, were healthy, and were free of obstetric and cardiovascular risk factors, (2) they were the products of full-term pregnancies (37-42 weeks gestation) without perinatal complications, (3) had birth weights between 2500-

4000 grams, and (4) had normal assessments by a pediatrician/neonatologist. The selection was based on a non-probability sampling sequence. Neonates whose mothers resided outside the metropolitan area of Bucaramanga were excluded from the study to ensure lower losses in the follow-up. After exclusion of 715 neonates who did not meet the criteria, 154 neonates were invited to participate in the study, and 34 did not agree to participate. From these 120 neonates who underwent the first ECG record in the first 24 hours after birth (1st day), 113 returned for the one-week ECG (7th day), and 112 for the one-month ECG (30th day) (Figure 1).

Data collection

Neonatal electrodes were used to record the ECGs. Researchers involved were trained to standardize the location of precordial and limb electrodes. All precordial leads (V1 to V6 and V3R) as well as DI and DII leads were recorded directly. The remaining leads (DIII, aVR, aVL, aVF) were obtained from mathematical analysis of DI and DII.

ECG records were obtained in the morning, without sedation and in the mother's presence. Using a standardized protocol, ECGs were recorded in the first 24 hours (between 12-24 hours post-birth), in a week (7th day), and in a month (30th day) after birth. ECG signals were recorded using the digital BIOPAC MP35 (Biopac Systems USA) device, following the current recommended range (1200Hz sampling rate and 300Hz bandwidth)[7]. In addition, a 60Hz filter was applied to eliminate interference from AC power.

Neo 1.0 software, designed by CEMOS (Control, Electronics, Modeling, and Simulation – Universidad Industrial de Santander)[8], was used for reading electrocardiographic tracing. It measured the duration and amplitude of all waves and segments from the detection of singularities and characteristic points obtained by the wavelet transform and taking the PR segment as baseline. Neo 1.0 allowed viewing of all electrocardiography segments, intervals, and waves along with the start and end points of each, which were used to measure the duration and amplitude of all electrocardiographic parameters. This facilitates review and manual editing of each signal, enables the operator to relocate the point detection, and eliminates interference in the measurement of the digital signal.

Validation software Neo 1.0

Neo 1.0 software validation was used in two stages. In the first stage, the software's performance to adequately detect each of the singularities and characteristic points of electrocardiographic tracing was measured. A random sample of 100 ECG tracings was compared with the readings by a medical expert in electrocardiography. A sensitivity of 88.7% and a positive predictive value (PPV) of 94.3% was obtained for the P wave. Corresponding sensitivity and PPV were 99% and 99.2% for the QRS complex and 83.7% and 97.5% for the T wave respectively. In the second validation stage, duration and amplitude of electrocardiographic measurements were compared by both Neo 1.0 software and a pediatric cardiologist. To do so, we randomly selected 30 electrocardiographic signals. No statistically significant differences were found between the two readings.

Ethics

All parents of the neonates gave written informed consent. The study was approved by the research ethics committee of the Faculty of Health, Universidad Industrial de Santander, Bucaramanga, Colombia.

Statistical Analysis

In the descriptive analyses, continuous variables with normal distribution were reported as mean and standard deviations; otherwise, median and interquartile ranges were reported. Categorical variables were reported as percentages. For each of the ECG parameters, a longitudinal data analysis was performed using fixed (marginal) and random (conditional) effects models[9-11]. The distribution of the outcomes (each ECG parameter) was initially assessed for normality by a graphical analysis using histograms, box plots, and QQ plots. Covariates were adjusted for in the model and selected based on previous knowledge and publications which included: sex, birth weight, type of delivery (cesarean vs vaginal), gestational age, mother´s age, and number of gestations (including the current delivery). Time was categorized based on three time points for the ECG measurements (1st day, 7th day, and 30th day) and the first ECG registration (1st day) was considered as a reference. Interaction terms between time and sex as well as time and type of delivery were evaluated. For normally distributed outcomes, the restrictive estimation maximum likelihood model (REML) was used to select the best correlation matrix. To select the covariates to retain into the model, maximum likelihood estimation (MLE)

was used. Selection of the best model was based on the lowest value of Akaike information criterion (AIC) for outcomes with a normal distribution. A backwards manual selection procedure was used to arrive at the final model for each ECG parameter. Sex was retained in all models. Random intercept and random slope were evaluated for the conditional models. If the outcome was not normally distributed, a generalized estimation equation model (GEE) with unstructured matrix was used. A residual analysis was made for all final models. STATA version 14 was used for the statistical analysis[12] (Figure 2).

Results

Of the 120 neonates included, 64 were male (53.3 %) and 56 were female (46.7 %). 35% of the neonates weighed between 2,500 and 3,000 grams, 45.8% between 3,001 and 3,500 grams, and 19.2% between 3,501 and 4,000 grams. Method of delivery was Cesarean section for 51 neonates (42.5 %) and vaginal delivery for the remaining 69 neonates (57.5%). The reasons to perform Cesarean sections are described in Table 1. All the mothers underwent fetal monitoring during labor and were all normal.

Twenty electrocardiographic variables were analyzed in the 13 leads under study. Below, the analyses of the electrocardiographic parameters are presented. ECG findings in the first 24 hours after birth are the reference group against which the findings of the 7th and 30th days are compared.

Changes in ECG parameters and factors associated

AQRS Axis

The electrical axis at birth is shifted to the right, on average 128 degrees (SD±27.4). A linear mixed model, with both random intercept and slope adjusted for sex, showed that the electrical axis slightly increased by 6.48 degrees (CI95% 0.82 to 12.15) on the 7th day post-birth and it significantly reduced by 21.61 degrees (95% CI -28.09 to -15.13) on the 30th day post-birth. In addition, we found a significant association between the greater the number of pregnancies of the mother and an increase in the electrical axis (Beta: 4.76, 95% CI 1.57 to 7.95) (Table 2 and S1, Figure S1).

Heart Rate (DII)

The mean heart rate was 127.06 beats per minute (SD±16.8) at birth. A linear mixed model with both random intercept and slope adjusted for sex showed an increase of 12.55 beats per minute (95% CI 8.77 to 16.32) on the 7th day post-birth and an increase of 21.05 beats per minute (95% CI 17.21 to 24.88) on the 30th day post-birth (Table 2 and S1, Figure S2).

P wave duration (DII)

On average, wave duration in DII was 51.1 milliseconds (msec; SD±6.5) at birth. A mixed model with random intercept adjusted for sex showed no significant changes on the 7th day post-birth. By contrast, a significant increase of 2.7 msec (95% CI 1.25 to 4.16) was observed on the 30th day post-birth (Table 2 and S1, Figure S3).

PR Interval (DII)

Average PR interval duration at birth was 88.41 msec (SD±14.46). A linear mixed effects model with random intercept adjusted for sex showed a significant decrease of 4.77 msec (95% CI -8.17 to -1.37) in the PR interval on the 7th day post-birth. In addition, we found a significant association between birth weight (Beta: 0.0065, 95% CI 0.0016 to 0.011) and the mother´s age (Beta: 0.396, 95% CI 0.065 to 0.726) with an increase in PR interval (Table 2 and S1, Figure S4).

QRS duration (V5)

Mean duration of QRS complex at birth was 62.42 msec (SD±8.38). A GEE linear model adjusted for sex showed a downward trend in this parameter over time. This decline was not significant on the 7th day post-birth but a significant decrease of 4.67 msec (95% CI -6.91 to -2.43) on the 30th day post-birth was observed (Table 2 and S1, Figure S5).

QTc Interval (V3R)

On average, QTc interval duration at birth was 451.86 msec (SD±34.16) with a range between 368 and 526 msec. A mixed-effects model with random intercept

and slope, adjusted for sex and type of delivery, showed a decrease of 14.72 msec (95% CI -24.52 to 4.91) on the 7th day post-birth and 21.9 msec (95% CI -30.15 to -13.66) on the 30th day post-birth. Cesarean section was significantly associated with an average increase of 8.95 msec (95% CI 1.08 to 16.82) in QTc Interval (Table 2 and S1, Figure S6).

P wave amplitude (DII)

At birth, the P wave had an average amplitude of 0.13 millivolts (mv; SD±0.08). In a linear GEE model adjusted for sex, P wave amplitude significantly increased by 0.097 mv (95% CI 0.057 to 0.136) on the 7th day and by 0.051 mv (95% CI 0.026 to 0.076) on the 30th day post-birth. We also observed a significant interaction between P wave amplitude with sex on the 7th day. Compared with female neonates, male neonates had a significant decrease of 0.071 mv (95% CI -0.12 to -0.020) in the P wave amplitude 7 days post-birth (Table 2 and S1, Figure S7).

Q wave amplitude (DIII)

Q wave average in DIII was 0.276 mv (SD±0.168) at birth. No significant changes in Q wave amplitude during the neonatal period were found. However, male infants showed an average decrease of 0.044 mv (95% CI -0.089 to -0.00048) in Q wave amplitude compared with female infants during the neonatal period. We also found birth weight to be significantly associated with an increase in average Q wave amplitude (Beta: 0.000264, 95% CI 0.00000462 to 0.0000482; Table No.2 and S1, Figure S8)**.**

R wave amplitude (V1, V5)

R wave in V1 had an average of 1.66 mv (SD±0.75) at birth. A mixed model with random intercept adjusted for sex showed no significant R wave amplitude increase by the 7th day, but a significant decline of 0.39 mv (95% CI -0.57 to -0.21) on the 30th day post-birth (Table 2 and S1, Figure S9).

Average R wave in V5 was 0.68 mv (SD±0.3) at birth. A mixed linear model with random intercept and slope adjusted for sex showed a significant increase of 0.099 mv (95% CI 0.037 to 0.16) on the 7th day and of 0.17 mv (95% CI 0.079 to 0.26) on the 30th day post-birth (Table 2 and S1, Figure S10).

S wave amplitude (V1, V5)

S wave in V1 had a mean amplitude of 1.13 mv (SD±0.58) at birth. A GEE model with unstructured matrix adjusted for sex showed a significant decrease of 0.126 mv (95% CI -0.25 to -0.005) on the 7th day and of 0.437 mv (95% CI -0.56 to -0.3) on the 30th day post-birth (Table 2 and S1, Figure S11).

Mean S wave in V5 was 1.05 mv (SD±0.42) at birth. A mixed linear model with random intercept adjusted for sex showed a significant decrease of 0.098 mv (95% CI -0.18 to - 0.0065) on the 7th day and a further decline of 0.27 mv (95% CI -0.36 to -0.17) on the 30th day post-birth (Table 2 and S1, Figure S12).

R/S index (V1, V5)

At birth, R/S index in V1 was predominantly positive, with an average of 1.56 mv (SD±0.76). A GEE model with unstructured matrix adjusted for sex, showed a significant increase of 0.26 mv (95% CI 0.08 to 0.43) on the 7th day and of 0.38 mv (95% CI 0.20 to 0.55) on the 30th day post-birth (Table 2 and S1, Figure S13).

Mean R/S index in V5 was 0.69 mv (SD±0.33) at birth. A GEE model with unstructured matrix adjusted for sex showed a significant gradual upward trend in R/S index during the neonatal period, with an increase of 0.16 mv (95% CI 0.10 to 0.23) on the 7th day and of 0.49 mv (95% CI 0.42 to 0.57) on the 30th day post-birth (Table 2 and S1, Figure S14).

Polarity of T wave (V1, V2)

We observed a predominance of positive T waves in the right-sided heart leads (72.28% and 83.17% for V1 and V2 respectively) in the first 24 hours. On the 7th day post-birth, the biphasic (positive/negative) waveforms were predominant (55.24% and 31.11% for V1 and V2 respectively). A clear predominance of negative waves (80.19% and 62.82% for V1 and V2 respectively) was observed on the 30th day post-birth. However, a significant percentage of remnant biphasic (negative/positive) wave forms persisted till the 30th day post-birth (18.87% and 16.67% for V1 and V2 respectively; Table 3).

Polarity of T wave (V5, V6)

Polarity of the T wave in V5 and V6 was predominantly positive (70.83% and 67.35% respectively) within 24 hours of birth. Its positivity increased to 74.19% and 78.13%, respectively, on the 7th day. On the 30th day post-birth, the majority was positive (99.2% and 97.2% respectively; Table 3). These findings are similar to those reported in previous studies.[13-16]

Discussion

We evaluated electrocardiographic dynamic changes and their associated factors in a cohort of healthy newborns followed during the neonatal period. Our results show that electrocardiographic parameters in the neonate are prone to change dynamically within the first month of life and factors related to the mother, the delivery and the newborn can play a role in these changes.

We observed a slight increase in the electrical AQRS axis on the 7th day after birth and decrease on the 30th day, compared to the first 24 hours after birth. Heart rate, P wave amplitude in DII, and R wave amplitude in V5 increased significantly on the 7th and the 30th day. PR interval decreased significantly on the 7th day. QTc Interval in V3R and S amplitude in V1 and in V5 decreased significantly on both the 7th and the 30th day ECGs. QRS duration in V5 and R amplitude in V1 significantly decreased on the 30th day. R/S ratio in V1 increased on the 7th and 30th day ECGs but the increase was smaller compared with the R/S ratio in V5.

We also found certain characteristics of the newborn and the mother as well as the type of delivery to be associated with subsequent changes in neonatal electrocardiogram. Weight at birth was associated with an increase in average PR interval in DII and average amplitude of Q wave in DIII. Maternal factors such as the number of pregnancies were positively associated with an average increased electrical axis and maternal age was positively associated with an average increased PR interval. Cesarean section was associated with a significant increase in average QTC interval in V3R. Moreover, we observed a significant interaction between sex of neonate and P wave amplitude of DII on the 7th day after birth with a lower P-wave amplitude in male neonates compared with female neonates. The biological plausibility of these findings, however, is not straightforward and these results need to be confirmed in future studies.

When comparing our results with those reported by Davignon et al.[5], we found statistically significant differences in the duration and amplitude of 88% of all recorded electrocardiographic variables, except for the QRS axis on the 7th day, R wave amplitude in V1 on the 30th day, and amplitude of S wave in V5 during the whole neonatal period. These differences can be explained in part by the different techniques of electrocardiography recorded since Davignon et al.[5] used a much lower sampling frequency (333 Hz) compared to our study. The currently recommended sampling rate of 1200 Hz and minimum bandwidth 250 Hz, used in our study, are based on the report by Rijnbeek et al.[6,7], which showed that higher sampling rate and larger bandwidth are required to adequately capture the different frequency bands of ECG in pediatric electrocardiography.

The trajectories of the waves and electrocardiographic intervals during the neonatal period in our study followed a similar trend described by Davignon et al.[5], with a concordance of 82% (table S1). The differences between the trajectories in our study and the report by Davignon et al.[5] includes a slight increase in the electrical axis on the 7th day, an increase in the PR interval in DII from the 7th to the 30th day, a decrease in the QRS complex duration in V5 on the 30th day after birth, and an increase in the R/S ratio in V5 during the neonatal period in our study.

Our findings are in line with previous reports: (1) Furman et al.[13] showed neonates with AQRS axis higher than 120 grades a week post-birth and Datey et al.[14] described AQRS axis higher than 125 grades at a week post-birth; (2) Michaelsson et al.[15] reported a slight, non-significant, increase in PR interval in the first month; (3) Ziegler et al.[16] showed dynamics changes in T waves similar to our findings; and (4) Sutin et al.[17] paid attention to ethnicity differences[17] that could produce changes in some ECG parameters.

Other studies, such as Macfarlane et al.[18,19], used a sampling rate of 500 Hz in a Caucasian population and showed that the upper 98 percentile limit of normal amplitudes in ECG could be up to 46% higher and the duration of QRS could be wider compared with Davignon's results[5]. Similar to our findings, their study showed a decrease in the tendency of S wave amplitude and a decrease in QRS duration among male neonates one month after birth [18,19]. Rijnbeek et al.[6], using a sampling rate of 1200 Hz among 1912 Dutch children aged 11 days to 16 years, showed higher median values compared with Davignon´s results. These results, however, may not be directly comparable to our findings as they combined all the newborns between 11 to 30 days in one group.

4.1 Strengths and Limitations

Our study is a cohort study following the same group of newborns during the whole neonatal period with a small proportion (6.7%) lost to follow-up. Our results were based on linear regression models for correlated data. This analysis produces mathematical equations that help predict the value of each electrocardiographic parameter in an easier and more valid way, thus avoiding the use of graphics and tables that are more complicated to interpret. Other studies had reported linear models based on cross-sectional data, limited to a few electrocardiographic variables[18] and with relatively small sample sizes in the neonatal period[6,20]. We used a digital computerized register following the current recommendations of sampling rate (1200 HZ) and bandwidth (300 Hz) and standardized the location of the electrodes to ensure the validity of our results. Moreover, we used an internally validated software to read all waves, segments, and intervals in each ECG. Having access to detailed information, we performed longitudinal data analysis of repeated ECG parameters to evaluate the significant changes over time adjusted for the characteristics of the newborn, the mother, and the delivery method.

The limitations of our study also merit consideration. The included newborns in our study were considered healthy based on their (term) gestational age, birth weight, lack of fetal or maternal complications, and clinical evaluation by a neonatologist. Our cohort was of only Hispanic-American newborns enrolled at a single hospital center, a fact that limits the generalizability of our findings. Although our Neo 1.0 software was internally validated, external validity of this software as well as comparison of its performance with other available pediatric electrocardiography software is desirable. While our sample size was sufficient to show significant changes in ECG parameters during the neonatal period, it might have been limited to adequately assess and address gender differences.

4.2 Clinical Implications and Directions for Future Research

The existing evidence that forms the basis for the reference standard values for neonatal electrocardiogram in the current guidelines is mainly based on single-center, cross-sectional studies. Our results highlight the need to move towards a consensus on the dynamics of electrocardiographic changes in the neonatal period. To this end, multi-center and multi-ethnic studies with electrocardiographic computerized records according to current recommendations[7] and available follow-up during the neonatal period in the same cohorts are essential. Such studies

would allow for proper statistical analysis of repeated ECG measures as well as disentangling potential sex and racial differences.

4.3 Conclusions

Our study outlines the dynamics of electrocardiographic parameters during the neonatal period in a cohort of healthy newborns. We also found certain characteristics of the newborn and the mother as well as the type of delivery to be associated with subsequent changes in neonatal electrocardiogram. Our results highlight the need to move towards a consensus on the dynamics of electrocardiographic changes in the neonatal period by conducting multi-center and multi-ethnic prospective cohort studies with electrocardiographic computerized records according to current recommendations.

Conflict of interest

None declared

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Fig. 1 Flow-chart for inclusion of the study participants

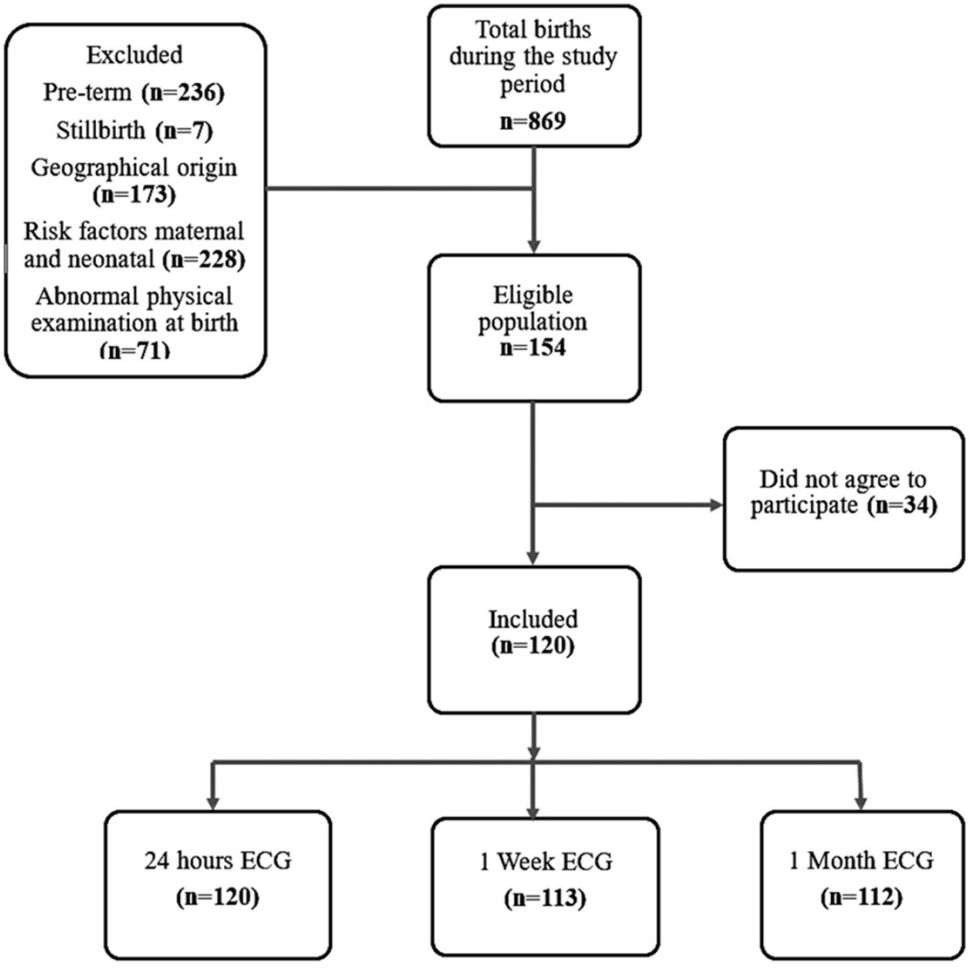


Fig. 1 Flow-chart for inclusion of study participants

Table 1. Characteristics of the newborns included in the study (n=120)

Characteristics	Value
Sex (Male)	64 (53.3)
Weight at birth, grams	3185 (2805-3430)
Age of mother, years	23 (19-27)
Gestational age, weeks	39 (38-40)
Number of gestations	2 (1-3)
Delivery method	
Vaginal delivery	69 (57.5)
Caesarean section	51 (42.5)
Reason for undergoing caesarean section	
Previous caesarean	26 (51.0)
Cephalopelvic disproportion	13 (25.5)
Labor arrest	6 (11.7)
Breech delivery	3 (5.8)
Transverse delivery	1 (2.0)
Condilomatosis	1 (2.0)
Valuable fetus	1 (2.0)

Values are for number (%) or median **(**first quartile - third quartile).

Table 2. Changes over time in neonatal electrocardiographic variables using correlated linear models in (n=120)

Variable	n	Model	Constant β CI 95%	7 Days β CI 95%	30 Days β CI 95%	Sex (Male) β CI 95%
QRS Axis [1]	299	Mixed-2	114.94 ‡ (104.7, 125.16)	**6.48 *** **(0.82, 12.15)**	-21.61 ‡ (-28.09, -15.13)	4.54 (-3.56, 12.65)
Heart Rate DII	327	Mixed-2	128.24 ‡ (124.9, 131.6)	12.55 ‡ (8.77, 16.32)	21.05 ‡ (17.21, 24.88)	-2.13 (-5.6, 1.34)
Duration P DII	300	Mixed-1	50.81 ‡ (49.23, 52.40)	-0.652 (-2.13, 0.82)	2.70 ‡ (1.25, 4.16)	0.48 (-1.34, 2.3)
PR Interval DII[2]	310	Mixed-1	57.72 ‡ (40.65, 74.78)	-4.77 ‡ (-8.17, -1.37)	**-1.09** **(-4.5, 2.3)**	1.62 (-2.26, 5.49)
QRS Duration V5	325	GEE	61.74 (59.89, 63.59)	- 1.89 (-3.88, 0.09)	**-4.67 ‡** **(-6.91, -2.43)**	1.45 (-0.47, 3.38)
QTc Duration V3R[3]	284	Mixed-2	**448.35 ‡** **(440.5, 456.15)**	-14.72 ‡ (-24.5, -4.91)	-21.9 ‡ (-30.1, -13.66)	-0.42 (-8.2, 7.38)
P Amplitude DII[4]	279	GEE	0.128 ‡ (0.09, 0.15)	0.097 ‡ (0.057, 0.136)	**0.051 ‡** **(0.026, 0.076)**	0.006 (-0.026, 0.039)
Amplitude Q DIII[5]	313	GEE	0.056 (-0.118, 0.23)	0.0216 (-0.020, - 0.063)	- 0.021 (0.068, -0.025)	**-0.044 *** **(-0.089 , -0.0004)**
R V1 Amplitude	327	Mixed-1	1.67 ‡ (1.5, 1.83)	**0.14** **(-0.04, 0.32)**	-0.39 ‡ (-0.57, -0.21)	-0.02 (-0.19, 0.15)

R V5 Amplitude	325	Mixed-2	0.73 ‡ (0.66, 0.8)	0.099 ‡ (0.037, 0.16)	0.17 ‡ (0.079, 0.26)	-0.08 (-0.16, 0.006)
S V1 Amplitude	319	GEE	1.17 ‡ (1.048, 1.29)	-0.126 * (-0.25, -0.005)	-0.43 ‡ (-0.56, -0.30)	-0.06 (-0.20, 0.08)
S V5 Amplitude	329	Mixed-1	1.09 ‡ (1.0, 1.18)	-0.098 * (-0.18, -0.006)	-0.27 ‡ (-0.36, -0.17)	-0.07 (-0.17, 0.024)
R/S V1	311	GEE	1.58 ‡ (1.4, 1.75)	0.258 ‡ (0.082, 0.43)	0.379 ‡ (0.20, 0.55)	-0.002 (-0.20, 0.20)
R/S V5	324	GEE	0.705 ‡ (0.62, 0.79)	**0.164 ‡** **(0.10, 0.23)**	**0.49 ‡** **(0.42, 0.57)**	-0.0045 (-0.109, 0.10)

GEE: Generalized Estimator Equation model, Mixed-1: Mixed effect model with random intercept, Mixed-2: Mixed effect model with both intercept and slope random

*P<0.05; ‡p< 0.01.

Bold type denotes main differences in tendencies compared with the report by Davignon[5].

[1]Adjusted for number of gestations (Beta: 4.76 CI95% 1.57, 7.95 p=0.003)

[2]Adjusted for birth weight (Beta: 0.0065 CI95% 0.0016, 0.011 p=0.009)

Adjusted for mother´s age (Beta: 0.396 CI95% 0.065, 0.726 p=0.019)

[3]Adjusted for Caesarean section (Beta: 8.95 CI95% 1.08, 16.82 P= 0.026)

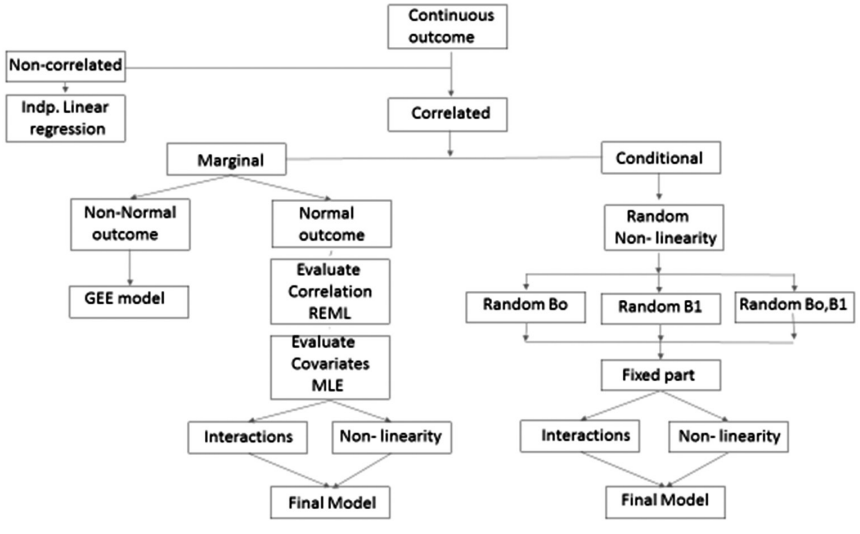
[4]Adjusted for time7sex (Beta: -0.071 CI95% -0.12, -0.020 p=0.006)

[5]Adjusted for birth weight (Beta= 0.000264 CI95% 0.0000046, 0.0000482, P= 0.018)

Table 3. Changes in T wave polarity during the neonatal period.

Derivations	n	% T Plane	% T (+)	% T (-)	% T (+/-)	% T (-/+)
V1, at birth	101	---	72.28	13.86	0.99	12.87
V1, 7 th day	105	0.95	6.67	37.14	---	55.24
V1, 30th day	106	---	---	80.19	0.94	18.87
V2, at birth	101	4.95	83.17	8.91	---	2.97
V2, 7 th day	90	7.78	17.78	42.22	1.11	31.11
V2, 30th day	78	1.28	15.38	62.82	3.85	16.67
V5, at birth	96	3.13	70.83	12.5	9.38	4.17
V5, 7 th day	93	---	74.19	3.23	21.51	1.08
V5, 30th day	102	---	99.02	0.98	---	---
V6, at birth	98	7.14	67.35	10.2	15.31	---
V6, 7 th day	96	---	78.13	2.08	17.71	2.08
V6, 30th day	107	---	97.2	1.87	---	0.93

Figure 2. Flow chart of longitudinal data analysis



Indp: Independent GEE: Generalized estimator equation model REML: Restricted maximum likelihood MLE: Maximum likelihood estimation Bo: Beta Intercept B1: Beta Slope

Table S1. Comparison of changes over time in the trajectories of electrocardiographic variables in neonatal period between Davignon´s report and our findings

Davignon							UIS-ERASMUS							
Heart rate DII														
Age	**N**	**Mean**	**Median**	**(p25)**	**(p75)**	**Tendency**	**N**	**Mean**	**Median**	**(p25)**	**(p75)**	**p-value**	**Tendency**	**Conclusion**
1 Day	189	121	122	113	133	Reference	111	127.0	125.0	114.8	138.2	0.008	Reference	Reference
7 Days	181	127	128	115	140	Increase	111	139.6	139.9	127.4	148.5	0.000	Increase	Concordant
30 Days	119	149	151	135	162	Increase	105	148.0	147.5	141.6	157.7	0.045	Increase	Concordant
P Wave duration (DII)														
1 Day	NA	NA	NA	NA	NA	Reference	95	51.1	51.7	46.0	55.8	NA	Refrence	Reference
7 Days	NA	NA	NA	NA	NA	NA	99	50.6	50.1	46.3	53.9	NA	Decrease	NA
30 Days	NA	NA	NA	NA	NA	NA	106	53.6	53.6	48.8	58.8	NA	Increase	NA
PR interval (DII)														
1 Day	188	---	105	95	117	Reference	101	88.4	86.7	78.4	100.7	0.000	Reference	Reference
7 Days	181	---	103	93	111	Decrease	105	83.8	82.8	73.6	93.7	0.000	Decrease	Concordant
30 Days	115	---	99	90	112	Decrease	104	87.4	86.4	78.0	95.3	0.000	Increase	**No concordant**
QRS duration (V5)														
1 Day	187	---	50	45	58	Reference	107	62.4	61.8	56.4	66.8	0.000	Reference	Reference
7 Days	180	---	49	45	55	Decrease	110	60.6	58.6	53.9	64.9	0.000	Decrease	Concordant
30 Days	117	---	52	44	60	Increase	108	57.9	55.8	52.0	61.0	0.000	Decrease	**No concordant**
QTc (V3R)														
1 Day	186	---	290	264	315	Reference	86	451.8	457.8	423.7	478.0	0.000	Reference	Reference
7 Days	177	---	273	255	285	Decrease	102	437.1	423.2	403.0	467.6	0.000	Decrease	Concordant
30 Days	117	---	258	248	273	Decrease	96	429.9	428.1	416.0	439.9	0.000	Decrease	Concordant
QRS Axis														
1 Day	189	135	135	115	160	Reference	102	128.0	127.0	109.0	143.0	0.002	Reference	Reference
7 Days	180	131	131	110	154	Decrease	96	135.0	140.0	117.0	152.0	0.128	Increase	**No concordant**
30 Days	117	110	110	95	120	Decrease	101	106.0	105.0	86.0	115.0	0.018	Decrease	Concondart

Davignon							UIS-ERASMUS							
P wave amplitude (DII)														
Age	**n**	**Mean**	**Median**	**(p25)**	**p75)**	**Tendency**	**n**	**Mean**	**Median**	**p25)**	**(p75)**	**p-value**	**Tendency**	**Conclusion**
1 Day	188	---	0.17	0.13	0.20	Reference	83	0.134	0.126	0.07	0.18	0.000	Reference	Reference
7 Days	180	---	0.18	0.13	0.22	Increase	98	0.191	0.177	0.118	0.26	0.000	Increase	Concordant
30 Days	119	---	0.19	0.15	0.23	Increase	98	0.184	0.159	0.096	0.28	0.000	Decrease	**No concordant**
Q Wave amplitude (DIII)														
1 Day	189	0.14	0.12	0.05	0.16	Reference	107	0.276	0.239	0.145	0.329	0.000	Reference	Reference
7 Days	181	0.16	0.14	0.07	0.25	Increase	104	0.298	0.274	0.18	0.42	0.000	Increase	Concordant
30 Days	119	0.15	0.13	0.06	0.25	Decrease	102	0.25	0.238	0.091	0.36	0.000	Decrease	Concordant
R Wave amplitude (V1)														
1 Day	189	---	1.3	1.1	1.72	Reference	113	1.661	1.568	1.089	2.142	0.000	Reference	Reference
7 Days	181	---	1.25	0.89	1.62	Decrease	107	1.802	1.790	1.294	2.166	0.000	Increase	**No concordant**
30 Days	119	---	1.14	0.71	1.37	Decrease	107	1.270	1.325	0.744	1.740	0.095	Descrease	Concordant
R Wave amplitude (V5)														
1 Day	189	---	1.0	0.7	1.4	Reference	112	0.683	0.647	0.439	0.884	0.000	Reference	Reference
7 Days	181	---	1.3	0.8	1.45	Increase	112	0.787	0.755	0.578	0.935	0.000	Increase	Concordant
30 Days	119	---	1.4	1.1	1.7	Increase	101	0.854	0.912	0.698	1.1448	0.000	Increase	Concordant
S Wave amplitude (V1)														
1 Day	189	---	0.78	0.47	1.2	Reference	108	1.132	0.966	0.703	1.451	0.000	Reference	Reference
7 Days	181	---	0.6	0.26	0.6	Decrease	107	1.027	0.960	0.575	1.332	0.000	Decrease	Concordant
30 Days	119	---	0.4	0.2	0.4	Decrease	104	0.711	0.712	0.330	0.984	0.000	Decrease	Concordant

Davignon							UIS-ERASMUS							
S Wave amplitude (V5)														
1 Day	187	---	0.98	0.65	1.3	Reference	111	1.055	1.027	0.713	1.420	0.145	Reference	Reference
7 Days	181	---	0.9	0.63	1.2	Decrease	110	0.955	0.959	0.701	1.233	0.117	Decrease	Concordant
30 Days	118	---	0.8	0.58	1.02	Decrease	108	0.785	0.787	0.578	1.055	0.907	Decrease	Concordant
R/SV1														
1 Day	178	---	1.8	1.2	2.6	Reference	110	1.565	1.402	1.037	2.017	0.000	Reference	Reference
7 Days	174	---	2.0	1.4	3.1	Increase	99	1.802	1.734	1.323	2.209	0.001	Increase	Concordant
30 Days	103	---	2.5	1.5	3.7	Increase	102	1.931	1.810	1.354	2.360	0.000	Increase	Concordant
R/SV5														
1 Day	187	---	2	2	4	Reference	111	0.691	0.610	0.453	0.824	0.000	Reference	Reference
7 Days	181	---	2	2	4	Equal	110	0.861	0.769	0.585	1.064	0.000	Increase	**No concordant**
30 Days	119	---	2	2	4	Equal	103	1.186	1.157	0.859	1.458	0.000	Increase	**No concordant**

Changes in amplitude and duration in the neonatal electrocardiogram

Figure S1. AQRS Axis

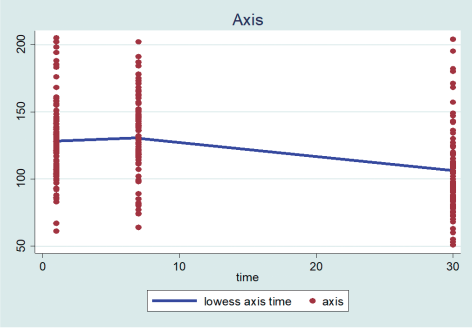


Figure S2. Heart Rate (DII)

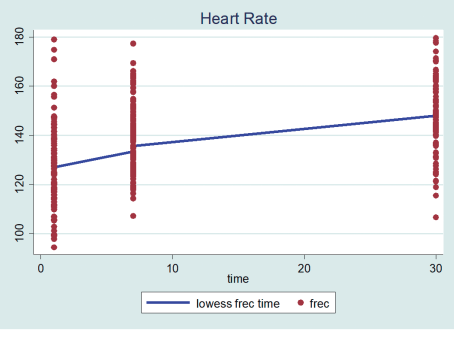


Figure S3. P wave duration (DII)

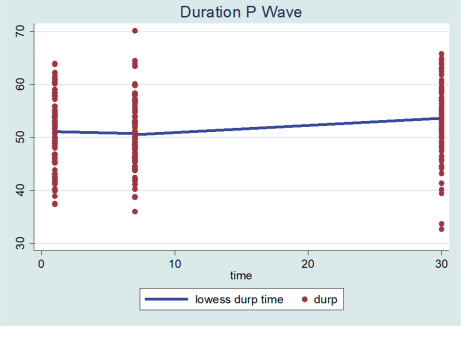


Figure S4. PR Interval (DII)

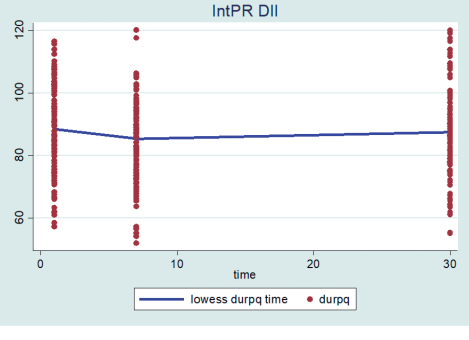


Figure S5. QRS duration (V5

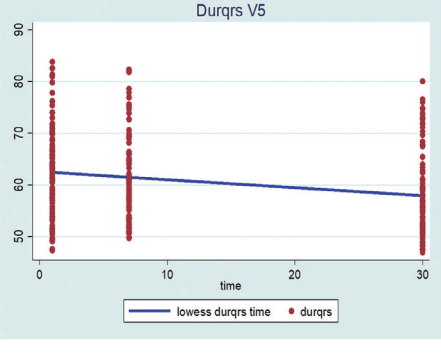


Figure S6. QTc Interval (V3R)

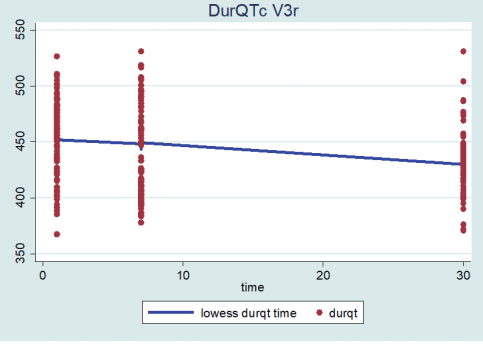


Figure S7. P wave amplitude (DII) by sex

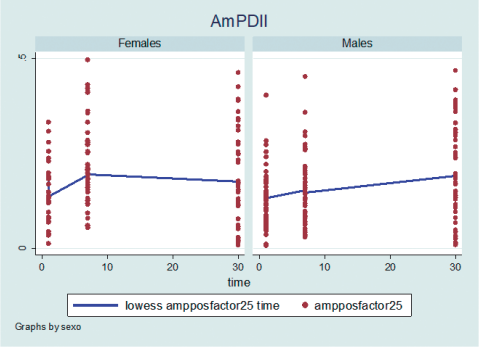


Figure S8. Q wave (DIII) by sex

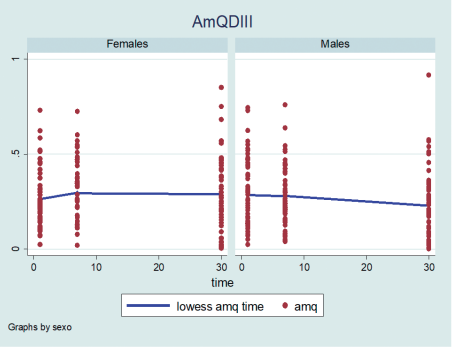


Figure S9. R wave amplitude (V1)

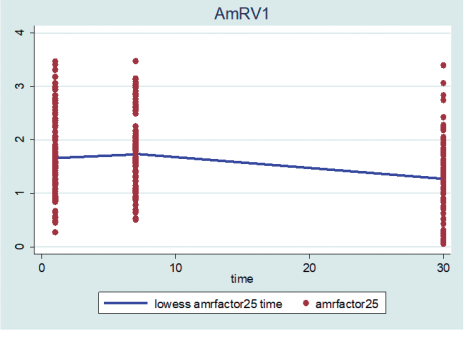


Figure S10. R wave amplitude (V5)

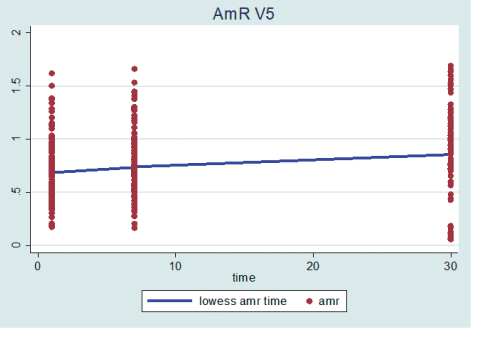


Figure S11. S wave amplitude (V1) (V5)

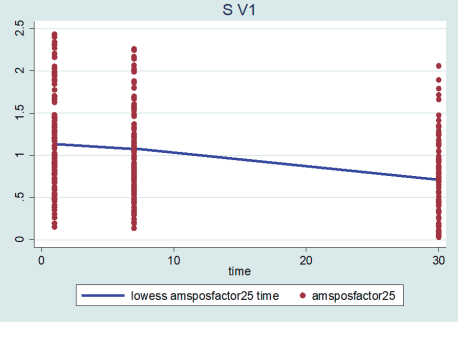


Figure S12. S wave amplitude

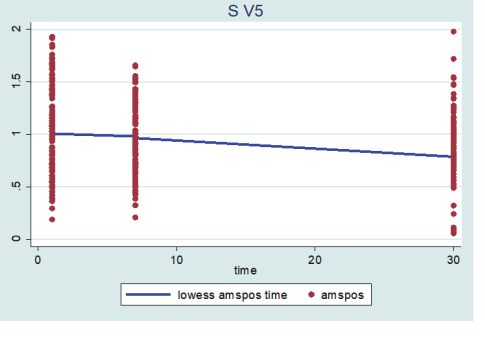


Figure S13. R/S index (V1)

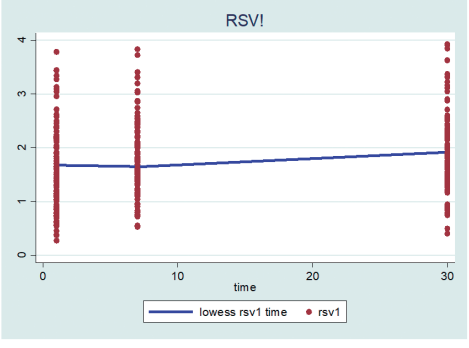
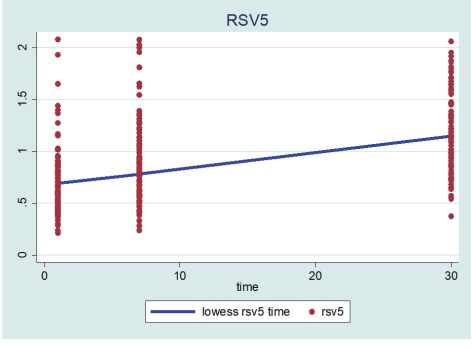


Figure S14. R/s index (V5)



CHAPTER 2.2

Risk factors for longitudinal changes in left ventricular diastolic function among women and men: The Rotterdam Study

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Abstract

Objective

To evaluate changes in left ventricular diastolic function (LVDF) parameters and their associated risk factors over a period of 11 years among community-dwelling women and men.

Methods

Echocardiography was performed three times among 870 women and 630 men (age 67±3 years) from the prospective population-based Rotterdam Study during a period of 11 years follow-up. Changes in six continuous LVDF parameters were correlated with cardiovascular risk factors using a linear-mixed effect model (LMM).

Results

In women, smoking was associated with deleterious longitudinal changes in DT (7.73; 2.56, 12.9) and high-density lipoprotein cholesterol was associated with improvement of septal e' (0.37; 0.13, 0.62) and E/e' ratio (-0.46; -0.84,-0.08) trajectories. Among men, diabetes was associated with deleterious longitudinal changes in A wave (3.83; 0.06,7.60), septal e' (-0.40; -0.70,-0.09) and E/e' ratio (0.60; 0.14,1.06) and body mass index was associated with deleterious longitudinal changes in A wave (1.25; 0.84,1.66), E/A ratio (-0.007; -0.01,-0.003), DT (0.86; 0.017, 1.71), and E/e' ratio (0.12; 0.06, 0.19).

Conclusions

Smoking among women and metabolic factors (DM and BMI) among men showed larger deleterious associations with longitudinal changes in LVDF parameters. The favorable association of HDL was mainly observed among women. This study, for the first time, evaluates risk factors associated with changes over time in continuous LVDF parameters among women and men and generates new hypothesis for further medical research.

Key words: Echocardiography, Doppler – Left ventricular diastolic function – Cohort study – Sex difference – risk factors

Key messages

What is already know about this subject?

Left ventricular diastolic dysfunction and HFpEF occurs more frequent in women than men but it is not clear what risk factors are associated with these gender differences.

What does this study add: Our results show the differential association of risk factors with longitudinal alterations in the LVDF parameters among women and men. We observed a larger deleterious association for smoking among women and for BMI and DM among men with longitudinal changes in LVDF parameters over time. The favorable association of HDL cholesterol with LVDF was more pronounced among women.

How might this impact on clinical practice?: Changes over time in the trajectories of continuous LVDF parameters among women and men and their associated risk factors provide a novel hypothesis platform for further medical research.

Introduction

Left ventricular diastolic dysfunction is highly prevalent and worsen with advancing age[1-3]. Persistence or progression of diastolic dysfunction is a risk factor for heart failure(HF) among the elderly[2]. Recent data suggest that diastolic dysfunction is present in the majority, around 70%, of patients with heart failure with preserved ejection fraction (HFpEF)[4]. Although plenty of evidence-based treatments for heart failure with reduced ejection fraction (HFrEF) exist, there is no treatment with proven benefits for HFpEF[5].

Impairment of left ventricular diastolic function(LVDF) occurs gradually and has been shown to be, at least partly, reversible[1,6], Therefore, early detection of subclinical impairment in LVDF and identification and treatment of its associated risk factors to prevent or slow the progression to overt HF is important. To date, several risk factors associated with LVDF have been identified[7,8]. However, longitudinal studies evaluating changes in continuous LVDF parameters over time in general population of subjects without clinically diagnosed HF are scant and have been mostly performed among middle-aged individuals. As occurrence of various HF phenotypes differs between women and men[5], it has been suggested that gender differences in susceptibility to risk factors might partly explain these dissimilarities[6]. However, recent studies have failed to address gender

differences in the setting of changes in LVDF and its associated risk factors[4-7]. Notably, while women tend to have a better LVDF until 60 years of age, gender disparities are reversed after the menopause[5]. To further clarify sex differences in the pathophysiology of diastolic dysfunction, studying changes in continuous LVDF parameters among women and men and their correlates, especially at older ages, is warranted.

We, therefore, aimed to evaluate longitudinal changes in continuous LVDF parameters during 11 years of follow-up among women and men from a large prospective population-based cohort[9]. Participants were all free from clinically diagnosed HF at the time of echocardiographic examinations and during follow-up. In addition, we investigated the risk factors associated with the changes in LVDF parameters among women and men.

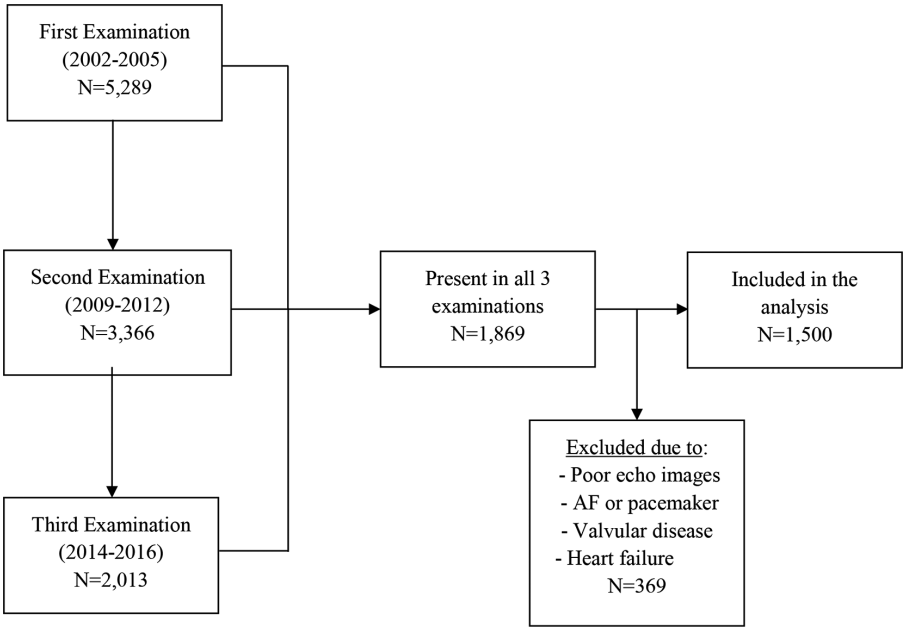
Methods

Study Population

The Rotterdam Study(RS) is a prospective population-based cohort that included participants aged 55 years and older in the district of Ommoord, Rotterdam, The Netherlands[9]. The study started in 1990 with 7,983 participants (RS-I) and was extended twice; in 2000 (RS-II, n=3,014) and in 2006 (RS-III, n=3,932). The follow-up examinations take place every 3-4 years. The RS was approved by the Medical Ethics Committee according to the Population Study Act Rotterdam Study. All participants provided written informed consent.

The present study used data for six LVDF echocardiographic parameters from the fourth, fifth, and sixth examinations of the first cohort (RS-I) and the second, third, and fourth examinations of the second cohort (RS-II). Out of the six LVDF parameters under study, three repeated echocardiographic measurements were available for four indexes among 1,869 participants. We excluded 369 individuals due to poor echocardiographic images, atrial fibrillation, artificial pacemaker, moderate-severe valve compromise, and clinically diagnosed HF at the time of echocardiographic examinations and during the follow-up. Therefore, we included a total of 1,500 participants (630 men and 870 women) (Figure 1). For two LVDF parameters, two repeated measurements were available in a total of 1,528 (646 men and 882 women) subjects from the fifth and sixth examinations of the first cohort (RS-I) and the third and fourth examinations of the second cohort (RS-II) (Online Figure 1).

Figure 1. Flowchart for the participants included in the analysis of longitudinal changes in LVDF parameters measured 3 times over 11 years of follow-up. AF, atrial fibrillation; LVDF, left ventricular diastolic function.



Left ventricular diastolic function parameters

We studied six continuous LVDF parameters. The apical 4-chamber view was used to measure the early trans-mitral ventricular diastolic filling velocity(E wave) and late diastolic filling velocity(A wave) during three cardiac cycles. Tissue Doppler imaging (TDI) was used to measure the early diastolic longitudinal filling velocity of the septal mitral annulus (septal e') during three cardiac cycles. The means of the E wave, A wave and septal e' over the three cardiac cycles were used to calculate E/A and E/e' ratios. Mitral valve deceleration time (DT) was measured as the time between the peak E-top wave and the upper deceleration slope extrapolated to the zero baseline using a Continuous Wave Doppler[10.11]. Additional information on echocardiographic measurements, is provided in the online-supplemental material.

Assessment of cardiovascular risk factors

Detailed information regarding the evaluation of cardiovascular risk factors is given in the online-supplemental material.

Statistical Analysis

In the descriptive analysis, continuous variables with normal distribution were reported as mean (standard deviations) and categorical variables as numbers (percentages). We compared the mean and percentage values for women and men using t-test and z-proportion tests respectively. Longitudinal changes in LVDF parameters over time were plotted, treating age as a time-varying covariate. For each of the six parameters, a longitudinal data analysis using a linear mixed effect model was performed. The outcome of interest in each model was the two or three repeated measurements for each index as a continuous variable. Systolic and diastolic blood pressure(SBP, DBP), heart rate (HR), total and high-density lipoprotein (HDL) cholesterol, blood pressure and lipid lowering medications (LLM), diabetes mellitus (DM), current smoking, previous coronary heart disease (CHD), left ventricular mass indexed by body surface area (LVM), left ventricular ejection fraction (LVEF), physical activity, left atrial diameter (LAD) and cohort were included in all models. Age was used as a time-varying covariate. All analyses were performed in total population and in women and men separately. We checked for possible interaction between sex and different covariates in the total population. We additionally checked for the interaction terms between age, as a time-varying, and all covariates. We also compared the characteristics of the included participants with those who did not return for the follow-up echocardiography examinations. For more details regarding the analyses consult the online supplemental material. The analyses were performed with R v.3.2.5 (R Foundation for Statistical Computing, Vienna, Austria), and STATA (version 14.0, Stata Corp, College Station, TX). A 2-sided P value of <0.05 was considered statistically significant. Additionally, we considered a more conservative Bonferroni corrected p value of <0.0083 ($= 0.05/6$, considering six LVDF parameters).

Results

Table 1 details the baseline characteristics of 870 women and 630 men for the analyses of E wave, A wave, E/A ratio, and DT, in whom 3 repeated measurements were available during 11.1 years of follow-up. Women had higher HR, total and HDL cholesterol, left atrial diameter (LAD) and ejection fraction. Men had larger DBP, LVM, left ventricular end-diastolic diameter (LVEDD), and left ventricular end-systolic diameter (LVESD) and CHD prevalence. For septal e' and E/e' ratio, two repeated measurements were available among 882 women and 646 men during 4.2 years of follow-up (Online Table 1).

Longitudinal changes in LVDF among women and men

Based on the plots for each statistical model, the shapes of the longitudinal changes in all six LVDF parameters over time were similar in women and men (Figure 2). There was not interaction between age (as a time-varying covariate) and sex. The plots revealed a progressive deleterious mono-directional change in the longitudinal trajectories of all six LVDF parameters over time; i.e. a gradual rise in E wave, A wave, DT and E/e´ ratio values and a gradual decline in E/A ratio and septal e'. Despite similar trends in LVDF changes in both sexes, there were statistically significant differences in the mean values, with overall poorer indexes in women. Online Table 2 presents detailed information on cross-sectional values for LVDF parameters per age and gender category.

Risk factors associated with longitudinal changes in LVDF

Since E wave, A wave, DT and E/e´ ratio values progressively, and deleteriously, raised over time, a positive Beta coefficient for a risk factor means that the risk factor was associated with increment in the trajectory of these LVDF parameters over time. On the contrary, a negative Beta coefficient means that the risk factor was associated with decrement in the trajectory of these LVDF parameters over time. Therefore, a positive risk factor coefficient is associated with an unfavorable progression and a negative risk factor coefficient into a favorable progression on LVDF parameters over time. E/A ratio and septal e' values progressively, and deleteriously, diminish over time. Therefore, a negative Beta coefficient for a risk factor means that the risk factors was associated with decrement and a positive coefficient means that the risk factor was associated with increment in the trajectory of these LVDF parameters over time. Therefore, a positive coefficient translates into a favorable progression and a negative coefficient into an unfavorable progression on LVDF parameters over time.

Table 2 and table 3 show all beta coefficients and confidence intervals of different risk factors with longitudinal changes in LVDF indexes over time among women and men. Online Tables 3 and 4 show the summary of the risk factors significantly associated with longitudinal changes in LVDF parameters among women and men. Figure 3 shows the core findings of our study, summarizing the main differences among women and men in risk factors associated with changes in LVDF trajectories.

E wave: Among both women and men, age and SBP were associated with rise in E wave while DBP and LVM were associated with decline in E wave over time.

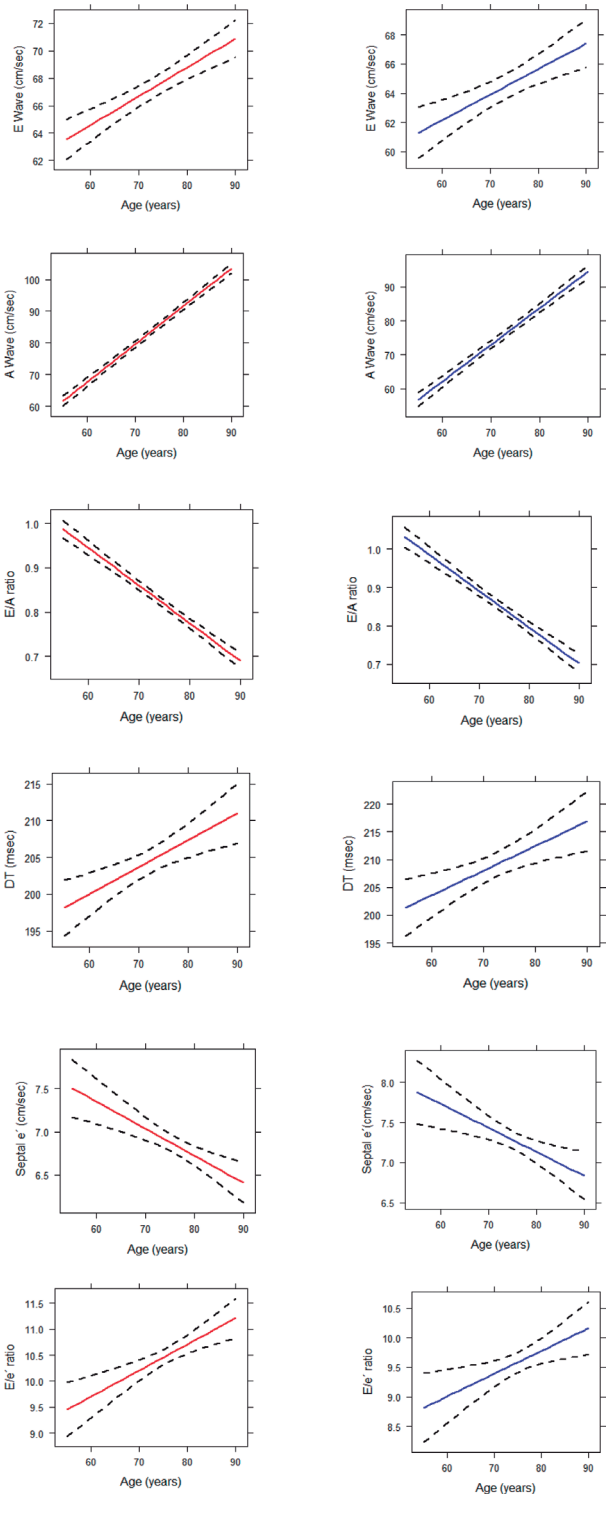
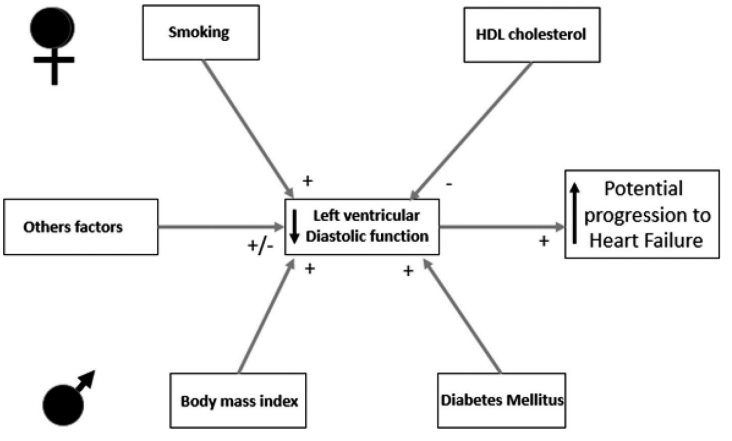


Figure 2 Plots for changes in LVDF parameters over time among women and men (red line: women, blue line: men). LVDF, left ventricular diastolic function.

Figure 3 The core findings of our study, showing the main risk factors associated with longitudinal changes in LVDF parameters among women and men. LVDF, left ventricular diastolic function.



Although BMI was associated with rise in E wave in both sexes, this association was only significant in women(Tables 2-3 & Online Tables 3-4).

A wave: Age, SBP, BMI and HR were associated with rise in A wave over time in both genders, and DM only in men (Tables 2-3 & Online Tables 3-4).

E/A ratio: Risk factors associated with decline in E/A ratio were age, DBP and HR in both genders. Only in men, BMI was significantly associated with decline in E/A ratio and LVEF and LAD with rise in E/A ratio(Tables 2-3 & Online Tables 3-4).

Deceleration Time: Among women, current smoking was the strongest risk factor significantly associated with rise in DT over time. Age was associated with rise in DT in both genders. SBP in women and HR in men were significantly associated with decline in DT. BMI was associated with rise in DT only in men (Tables 2-3 & Online Tables 3-4).

Septal e': LVM was associated with decline in septal e' in both genders. Additionally, LLM and prevalent of CHD among women and DM among men were associated with decline in septal e'. Among women, age and HDL Cholesterol were also associated with rise in septal e' (Tables 2-3 & Online Tables 3-4).

E/e' Ratio: LVM was associated with rise in E/e' ratio in both genders. Additionally, LLM was associated with rise and HDL cholesterol with decline in E/e' ratio

among women. Among men, prevalent CHD, BMI and DM were associated with rise in E/e' ratio(Tables 2-3 & Online Tables 3-4). P values for sex interaction in the associations of BMI and DM with E/e' ratio were significant.

Discussion

In the large prospective population-based Rotterdam Study, women had poorer diastolic function than men. However, the tendency of age-related changes in LVDF parameters over time was similar in both genders. Current smoking among women and metabolic factors such as BMI and DM among men were found to be associated with deleterious progression of longitudinal changes in LVDF parameters over time. HDL cholesterol showed a favorable association with LVDF trajectories mainly in women.

Although few studies have shown the intrinsic effect of age and several cardiovascular risk factors on worsening of LVDF parameters[3,7], a comprehensive longitudinal assessment of continuous LVDF parameters by gender over time is scant[6]. Patterns of longitudinal changes in the LVDF indexes over time in our study indicated a progressive impaired relaxation as well as increasing filling pressures with advancing age in both genders. In line with our findings, Kuznetsova et al[7] also found an rise in the E/e' ratio and decline in septal e' and E/A ratio over time. The LVDF parameters we reported are also comparable to those reported by Caballero et al[12] in populations older than 60 years, implying a worsening of diastolic function with ageing.

We found that the post-menopausal women in our study had a worse diastolic function compared to men, providing more evidence regarding the larger burden of diastolic dysfunction among women after menopause[5,12]. In younger men, a larger decline in most of the LVDF indexes over time was observed. Women have a better diastolic function until 60 years of age after which they experience a steeper decline and worse diastolic function compared to men[5]. Ageing per se seems to produce more eccentric remodeling and 3-fold larger apoptosis in men compared with women that might explain a steeper decline in diastolic reserve and the higher prevalence of diastolic dysfunction and HFpEF in women compared to men[13,14].

Longitudinal analyses of risk factors associated with changes in continuous LVDF parameters over time from a gender-specific perspective are scarce. Kuznetsova et al[7], based on the risk factors identified in cross-sectional studies, evaluated the longitudinal determinants of LVDF parameters and showed

advancing age, higher insulin levels, DBP, and HR to worsen LVDF indexes over time. A recent longitudinal analyses of Framingham[15], based on categorical LVDF parameters during 5.6 years follow-up, showed that age, female sex, changes in SBP and DBP, BMI, serum triglycerides and DM were associated with worsening diastolic function in total population. Our current study expands these findings by examining the risk factors associated with changes in various continuous LVDF parameters over 11 years of follow-up from a gender-specific perspective. The main advantage of analyzing the continuous LVDF parameters is a greater power to detect associations and a lower misclassification bias than analysis based on categorical classification[16].

Association of Risk Factors on Longitudinal Changes in LVDF parameters among Women and Men

Blood Pressure: SBP and DBP showed significant associations with longitudinal changes in E wave, A wave, and E/A ratio among women and men. The opposite direction of the effect for SBP and DBP suggested the effect of pulse pressure(PP). Accordingly, when we substituted SBP and DBP with PP in our analyses, PP was significantly associated with changes in these parameters among women and men. In several epidemiological studies, PP has shown a superior predictive value compared to SBP or DBP alone[17,18]. Higher PP is associated with elevated stress of the left ventricle which can result in ventricular hypertrophy and failure, critical determinants of left ventricular diastolic dysfunction[18].

Metabolic Factors: Previous cross-sectional studies have independently associated diastolic dysfunction with BMI and DM[19]. In our study, DM was found to be strongly associated with worsening of LVDF parameters in men. Expanded myocardial fibrosis as well as accelerated apoptosis are among the pathophysiologic features of diabetic cardiomyopathy[20]. While several previous studies have shown larger deterioration of LVDF among diabetics[8], data regarding sex differences in the association of DM on LVDF are scarce and conflicting. Diabetes was found to be an independent contributor to LVM among women in the Framingham Heart Study[21] but among both women and men from the Cardiovascular Health Study[22] and the Strong Heart Study[23].

In our study, a larger association of BMI with worsening of LVDF over time was found among men than in women. The only prior, cross-sectional, study that evaluated sex differences of obesity on LVDF, reported no association between BMI and LVDF indexes in women >65 years but did describe an association

between septal e' and abdominal adiposity among younger women Among men, BMI and abdominal obesity were associated with a higher likelihood of diastolic dysfunction[24]. The obesity-related mechanisms might be different for women and men. While for younger women the effect of obesity might act through its influence on SBP, the effect seems to be predominantly direct for men >65 years.

Smoking and Lipid Profile: Current smoking was only associated with rise in DT among women in our study. Smoking commonly precedes the development of HFpEF[25]. While smoking confers a greater CHD risk in women compared to men[26], sex differences in the setting of HF have not been reported[27]. Smoking has been suggested to significantly affect LVDF independently of its role as a risk factor for coronary atherosclerosis and through other independent pathways[28].

We found a favorable association of HDL-cholesterol with diastolic function over time among women. Moreover, use of lipid lowering medication, as a proxy for chronic dyslipidemia, was associated with worse LVDF over time. Previous cross-sectional studies have associated hyperlipidemia with coronary endothelial dysfunction and with myocardial damage independent of ischemia, leading to diastolic dysfunction[29]. Low levels of HDL cholesterol and elevated levels of total cholesterol are known risk factors for CHD and increasing LVM, both important factors leading to diastolic dysfunction. While increasing HDL levels have a more favorable effect in women compared to men, such gender differences in the association of HDL with LVDF require further study.

Study Strengths and Limitations

Our study was based on a large group of women and men from a population-based cohort with repeated echocardiographic examinations over 11 years of follow-up. The longitudinal design allowed the use of linear mixed effect models to analyze progressive long-term alterations in continuous LVDF parameters. Availability of the well-defined set of covariates and detailed characterization of the cohort allowed to examine LVDF parameters and their correlates from a gender-specific perspective. Nevertheless, limitations of our study also merit consideration. The gold standard for diastolic function measurement is the pressure-volume relationship which is an invasive approach. However, Doppler measurements of mitral inflow and TDI allows for a valid non-invasive measurement of diastolic function[10,30]. Echocardiography has proven to be a useful tool for assessing diastolic function, in order to minimize inherent limitations operator-dependent, a standardized protocol was used by 4 trained echocardiographers with good inter

and intra-reader agreement[11]. Our population included individuals of European ancestry. Therefore, the generalizability of our findings to other ethnicities should be performed with caution. As inherited to all longitudinal cohort studies, survival bias cannot be entirely ruled out.

Conclusions

In our large population-based study, women were found to have poorer diastolic function than men. However, age-related changes in continuous LVDF parameters were comparable in both genders. Our findings highlight the correlates of asymptomatic diastolic dysfunction among women and men. The differential association of risk factors with LVDF among women and men could provide further hypothesis regarding transition from a healthy heart to the development of HFpEF[5].

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Table 1. Baseline clinical and echocardiographic characteristics of the participants.

	Women (n=870)	Men (n=630)	p-value*
Clinical Features			
Age, years	67.30 (4.95)	67.29 (4.91)	0.980
BMI, kg/m²	27.42 (4.07)	27.08 (2.94)	0.069
SBP, mmHg	144.40 (18.32)	143.92 (19.20)	0.626
DBP, mmHg	79.84 (10.11)	82.01 (9.90)	<0.001
Blood Pressure Lowering Medication, n (%)	261 (30.0)	208 (33.0)	0.2130
Hypertension, n (%)	609 (70.0)	446 (70.8)	0.7378
Heart Rate, beats/min	69.36 (9.70)	65.79 (10.55)	<0.001
Total Cholesterol, mmol/L	5.96 (0.94)	5.45 (0.93)	<0.001
HDL-cholesterol, mmol/L	1.60 (0.40)	1.31 (0.31)	<0.001
Lipid Lowering Medication, n (%)	174 (20.0)	130 (20.63)	0.765
Current Smoker, n (%)	106 (12.2)	58 (9.2)	0.069
Prevalent CHD, n (%)	16 (1.84)	61 (9.68)	<0.001
Prevalent DM, n (%)	84 (9.66)	62 (9.84)	0.908
Echocardiography Features			
LVM index, g/m²	70.66 (15.47)	78.17 (18.19)	<0.001
Left Atrium Diameter/BSA, mm/m²	21.41 (2.69)	20.76 (2.45)	<0.001
LVEDD, mm	49.39 (4.96)	53.36 (4.86)	<0.001
LVESD, mm	30.12 (7.87)	33.66 (8.01)	<0.001
Relative Wall Thickness, cm	0.29 (0.06)	0.29 (0.05)	1
Ejection Fraction, %	65.87 (6.75)	63.69 (7.92)	<0.001
E wave cm/sec	67.38 (13.02)	64.48 (12.97)	<0.001
A wave cm/sec	83.33 (17.82)	76.61 (17.68)	<0.001
E/A ratio	0.83 (0.18)	0.86 (0.20)	<0.001
Deceleration time	204.4 (35.54)	209.19 (39.78)	<0.001
e`septal	6.87 (1.79)	7.29 (1.78)	<0.001
E/e`septal ratio	10.43 (2.62)	9.54 (2.51)	<0.001

* p-value for comparison of different characteristics between women and men.

Values are mean (± standard deviation) or numbers (percentages).

BMI: Body mass index, BSA: Body surface area, CHD: coronary heart disease, DBP: diastolic blood pressure, DM: Type 2 diabetes mellitus, LVEDD: Left ventricle end diastolic dimension, LVESD: Left ventricle end systolic dimension, LVM: Left ventricular mass, SBP: systolic blood pressure.

Table 2. Association of risk factors with longitudinal changes in left ventricular diastolic function parameters among women.

	E Wave	A Wave	E/A ratio	DT	Septal e'	E/e' ratio
Age*	4.43 (2.32, 6.53) †	1.22 (1.14, 1.30) †	- 0.43 (-0.47, -0.38) †	0.44 (0.20, 0.68) †	-0.02 (-0.005, -0.04) ‡	0.015 (-0.05, 0.019)
BMI	0.24 (0.04, 0.44) ‡	0.51 (0.25, 0.76) †	-0.0008 (-0.003, 0.002)	0.015 (-0.45, 0.48)	0.02 (-0.002, 0.05)	-0.015 (-0.06, 0.03)
SBP	0.11 (0.06, 0.17) †	0.18 (0.11, 0.24) †	-0.0002 (-0.0008, 0.0005)	-0.15 (-0.27, -0.03)‡	-0.006 (-0.01, 0.0009)	0.011 (-0.0005, 0.02)
DBP	-0.20 (-0.29, -0.11) †	-0.10 (-0.22, 0.01)	-0.002 † (-0.003, -0.0006)	0.10 (-0.11, 0.31)	-0.001 (-0.01, 0.01)	-0.007 (-0.03, 0.01)
BP lowering Medication	-1.63 (-3.40, 0.15)	-0.12 (-2.39, 2.16)	-0.01 (-0.03,0.01)	1.77 (-2.38, 5.91)	-0.22 (-0.44, 0.007)	0.27 (-0.074, 0.61)
Heart Rate	-0.02 (-0.10, 0.06)	0.37 (0.26, 0.47) †	-0.003 † (-0.004, -0.002)	-0.18 (-0.37, 0.005)	-0.01 (-0.02, 0.0006)	0.014 (-0.003, 0.03)
Total Cholesterol	0.009 (-0.80, 0.83)	-0.14 (-1.17, 0.89)	-0.0004 (-0.01, 0.01)	-0.62 (-2.51, 1.27)	-0.07 (-0.18, 0.032)	0.06 (-0.10, 0.23)
HDL-Cholesterol	1.19 (-0.73, 3.10)	-1.12 (-3.51, 1.27)	0.016 (-0.008, 0.04)	-3.70 (-8.07, 0.67)	0.37 (0.13, 0.62) †	-0.46 (-0.84, -0.08)‡
Lipid Lowering Medication	0.58 (-1.35, 2.50)	2.03 (-0.44, 4.49)	-0.001 (-0.03, 0.01)	-1.51 (-5.96, 2.95)	-0.28 (-0.54, -0.03)‡	0.55 (0.15, 0.96) †

	E Wave	A Wave	E/A ratio	DT	Septal e'	E/e' ratio
Current Smoking	-1.46 (-3.66, 0.74)	-0.43 (-3.20, 2.35)	-0.02 (-0.05, 0.01)	7.73 (2.56, 12.9) †	-0.18 (-0.51, 0.14)	-0.14 (-0.66, 0.37)
Left Ventricular Mass	-0.06 (-0.11, -0.01)‡	0.02 (-0.05, 0.08)	-0.0006 (-0.001, -0.0000003)	0.014 (-0.10, 0.13)	-0.02 (-0.03, -0.01) †	0.02 † (0.008, 0.03)
Prevalent CHD	-3.88 (-11.5, 3.69)	7.07 (-0.36, 14.51)	-0.06 (-0.14, 0.02)	-3.38 (-17.9, 11.22)	-0.56 (-1.07, -0.05)‡	0.84 (0.007, 1.68) ‡
Prevalent DM	1.38 (-1.20, 3.98)	1.72 (-1.50, 4.94)	-0.008 (-0.04, 0.02)	3.15 (-2.74, 9.03)	-0.03 (-0.31, 0.25)	-0.26 (-0.70, 0.18)
Ejection Fraction	0.07 (-0.04, 0.18)	0.06 (-0.09, 0.21)	0.001 (-0.0003, 0.003)	0.10 (-0.17, 0.38)	0.0007 (-0.013, 0.015)	0.011 (-0.011, 0.03)
Physical Activity	0.01 (-0.005, 0.03)	0.01 (-0.01, 0.04)	0.00005 (-0.0001, 0.0003)	-0.02 (-0.06, 0.02)	-0.002 (-0.004, 0.0005)	0.003 (-0.0005, 0.006)
Left Atrium Dimension	0.05 (-0.12, 0.21)	0.06 (-0.15, 0.28)	0.0004 (-0.001, 0.003)	-0.22 (-0.60, 0.17)	-0.0003 (-0.022, 0.021)	0.033 (-0.002, 0.07)

*Age in this analysis is used as a time-varying covariate.
†P< 0.01; ‡P<0.05. BMI: Body mass index, BP: blood pressure, CHD: Coronary heart disease, DBP: Diastolic blood pressure, DM: Diabetes Mellitus, HDL: High density lipoprotein, SBP: Systolic blood pressure.
Values are betas (95% confidence intervals).

Table 3. Association of risk factors with longitudinal changes in left ventricular diastolic function parameters among men.

	E Wave	A Wave	E/A ratio	DT	Septal e'	E/e' ratio
Age*	5.38 (2.60, 8.16) †	23.5 (20.4, 26.6) †	-0.010 (-0.012, -0.009) †	0.55 (0.23, 0.87) †	-0.006 (-0.02, 0.03)	0.015 (-0.05, 0.019)
BMI	0.22 (-0.10, 0.54)	1.25 (0.84, 1.66) †	-0.007 (-0.01, -0.003) †	0.86 (0.017, 1.71) ‡	-0.03 (-0.07, 0.006)	0.12 (0.06, 0.19) †
SBP	0.14 (0.08, 0.19) †	0.12 (0.05, 0.19) †	0.0001 (-0.0007, 0.0009)	-0.09 (-0.25, 0.05)	-0.002 (-0.01, 0.006)	0.028 (0.01, 0.04) †
DBP	-0.21 (-0.32, -0,10) †	-0.10 (-0.24, 0.03)	-0.002 (-0.003, -0.0004)‡	0.18 (-0.11, 0.46)	-0.005 (-0.02, 0.01)	-0.016 (-0.04, 0.008)
BP Lowering Medication	-0.78 (-2.79, 1.23)	1.90 (-0.66, 4.47)	-0.02 (-0.05, 0.005)	2.24 (-3.05, 7.53)	-0.09 (-0.33, 0.16)	0.11 (-0.27, 0.48)
Heart Rate	-0.10 (-0.18, 0.01)	0.21 (0.10, 0.32) †	-0.003 (-0.004, -0.002) †	-0.29 (-0.52, -0.06) ‡	0.003 (-0.007, 0.01)	-0.013 (-0.029, 0.003)
Total Cholesterol	-0.55 (-1.55, 0.45)	-0.45 (-1.75, 0.84)	0.013 (-0.0005, 0.03)	1.42 (-1.23, 4.06)	-0.12 (-0.25, 0.02)	0.022 (-0.18, 0.22)
HDL-Cholesterol	-0.28 (-3.07, 2.52)	1.79 (-1.85, 5.43)	-0.005 (-0.05, 0.03)	3.03 (-4.41, 10.47)	0.17 (-0.17, 0.51)	-0.04 (-0.56, 0.48)
Lipid Lowering Medication	0.23 (-2.11, 2.58)	-2.19 (-5.20, 0.83)	0.02 (-0.02, 0.05)	2.01 (-4.35, 8.38)	-0.15 (-0.45, 0.15)	-0.038 (-0.49, 0.41)

	E Wave	A Wave	E/A ratio	DT	Septal e'	E/e' ratio
Current Smoking	1.32 (-1.53, 4.16)	2.48 (-1.04, 6.0)	-0.007 (-0.05, 0.03)	2.72 (-4.79, 10.2)	0.04 (-0.39, 0.47)	-0.08 (-0.73, 0.57)
Left Ventricular Mass	-0.08 (-0,14, -0.03) †	-0.028 (-0.09, 0.04)	-0.0005 (-0.001, 0.0002)	0.006 (-0.13, 0.14)	-0.017 (-0.02, -0.01) †	0.017 (0.007, 0.03) †
Prevalent CHD	1.54 (-1.83, 4.91)	3.1 (-1.40, 7.54)	0.02 (-0.03, 0.06)	-1.59 (-10.96, 7.78)	-0.35 (-0.71, 0.023)	0.81 (0.26, 1.37) †
Prevalent DM	1.75 (-1.28, 4.77)	3.83 (0.06, 7.60) ‡	0.005 (-0.04, 0.05)	-1.09 (-8.94, 6.76)	-0.40 (-0.70, -0.09) ‡	0.60 (0.14, 1.06) ‡
Ejection Fraction	0.11 (-0.007, 0.22)	0.04 (-0.11, 0.18)	0.002 (0.0002, 0.003) ‡	0.28 (-0.023, 0.59)	0.004 (-0.01, 0.018)	0.011 (-0.012, 0.033)
Physical Activity	-0.005 (-0.03, 0.01)	-0.003 (-0.03, 0.02)	-0.00006 (-0.0004, 0.0002)	-0.02 (-0.07, 0.03)	0.002 (-0.0001, 0.005)	-0.003 (-0.007, 0.001)
Left Atrium Dimension	0.11 (-.0.09, 0.30)	-0.21 (-0.46, 0.04)	0.003 (0.0003, 0.006) ‡	-0.24 (-0.75, 0.27)	-0.002 (-0.02, 0.02)	-0.003 (-0.03, 0.02)

*Age in this analysis is used as a time-varying covariate.
†P< 0.01; ‡P<0.05. BMI: Body mass index, BP: blood pressure, CHD: Coronary heart disease, DBP: Diastolic blood pressure, DM: Diabetes Mellitus, HDL: High density lipoprotein, SBP: Systolic blood pressure.
Values are betas (95% confidence intervals).

SUPPLEMENTAL MATERIAL

Rueda-Ochoa OL, et al. Risk factors for longitudinal changes in left ventricular diastolic function among women and men: the Rotterdam Study.

Supplemental Methods

Echocardiography

Assessment of Cardiovascular Risk Factors

Statistical Analysis

Supplemental Results

Non-returning participants

Online Table 1. Baseline clinical and echocardiographic characteristics of the participants for the analysis of two left ventricular diastolic function parameters.

Online Table 2. Clinical and echocardiographic characteristics at the first examination for the individuals that participated only at the first examination and did not return for the two follow-up examinations.

Online Table 3. Association of risk factors with longitudinal changes in left ventricular diastolic function parameters among women.

Online Table 4. Association of risk factors with longitudinal changes in left ventricular diastolic function parameters among men.

Online Table 5. Left ventricular diastolic function parameters stratified by age and gender.

Online Figure 1. Flow chart for the participants included in the analysis of two left ventricular diastolic function parameters measured two times

References

Supplemental Methods

Echocardiography:

For each participant, one echocardiogram was obtained at each examination. In the first examination, the first 40% of the echocardiograms were performed with a commercially available ultrasonography system (AU3 Partner, Esaote Biomedica, with a 3.5/2.5 MHz transducer) and the followings with Acuson Cypress, with a 3V2c transducer. For the subsequent second and third examinations, a standardized protocol was used which also included two-dimensional resting transthoracic echocardiography performed by experienced echocardiographers with an identical standardized protocol for all participants and a commercially available ultrasonography system (Vivid I, GE Healthcare, Little Chalfont, UK), with a 2.5 MHz transducer. All examinations were performed by the same echocardiographers using the same protocol. As described previously, inter-reader and intra-reader agreements were good[1]. All images were digitally stored and assessed offline by the echocardiographers.

The protocol included 2-dimensional scanning in the parasternal long and short axis views, the apical and subcostal views. In addition, 2-dimension guided M-mode measurements of left ventricle were obtained by scanning in the parasternal long axis view.

Left atrial diameter (LAD), left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), interventricular septum thickness (IVST), left ventricular posterior wall thickness (LVPWT) and left ventricular ejection fraction were the left-sided measurements. Relative wall thickness was calculated according to the formula (2 * LVPWT)/(LVEDD) [2]. Left ventricular mass (LVmass) in grams was calculated according to the formula by Devereux and colleagues as 0.8 * (1.04 * ((LVEDD + IVST + LVPWT)3 - LVEDD3)) + 0.6 [3], and was indexed with Body Surface Area (BSA)[2]. Left ventricular fractional shortening (FS) was calculated using the formula: FS = (LVEDD-LVESD)/LVEDD * 100%[4].

Assessment of Cardiovascular Risk Factors:

Medical history, current health status, smoking and use of medications were assessed by a trained interviewer at the home visit using a computerized questionnaire. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice in sitting position at the right upper arm. We used the

average of two consecutive measurements. Hypertension was defined as SBP >140 mm Hg, DBP >90 mm Hg, or use of blood pressure-lowering medication with an indication for hypertension. Heart rate (HR) was measured with an oximeter in the second finger of the right hand and the average of two consecutive measurements was used. Total and high density lipoprotein (HDL) cholesterol and glucose levels were measured with the use of standardized laboratory techniques. Diabetes mellitus (DM) was defined as fasting glucose >6.9 mmol/L, nonfasting glucose >11.0 mmol/L, use of blood glucose-lowering medication, or a previous diagnosis of DM. A history of coronary heart disease (CHD) was defined as a myocardial infarction or coronary revascularization procedure[5, 6]. Physical activity was evaluated using LASA questionnaire (LAPAQ) and accelerometer (Actiwatch)[7].

Statistical Analysis:

For the analysis of the diastolic dysfunction indices, using each of the parameters as continuous variables, distribution of the outcome variable was graphically assessed for normality (histograms, box plots, and QQ plots). To select the correct function for the variable age (as a time-varying covariate), several different initial models including linear and non-linear functions together with interaction terms for age with other covariates were built. Outlier values were removed. The following covariates were included in the fixed part of the linear mixed models: Age (as time-varying covariate), systolic and diastolic blood pressure (SBP, DBP), heart rate (HR), total and high-density lipoprotein (HDL) cholesterol, blood pressure and lipid lowering medications, diabetes mellitus (DM), current smoking, previous coronary heart disease (CHD), left ventricular mass indexed by body surface area (LVM), left ventricular ejection fraction (LVEF), physical activity, left atrial diameter (LAD) and cohort. In the random part of the linear mixed model, age was the only variable (as a time-varying covariate) included. First, we evaluated a full model, including interactions terms, comparing model with random intercept vs model with both intercept and slope random. Second, we evaluated the linear and non-linear terms in the random part of the model and selected the model with lower AIC. Third, we evaluated the fixed part of the model, comparing full model with interactions terms vs model without interactions terms and selected the model with lower AIC. Finally, we evaluated the linear and non-linear terms in the fixed part of the model and selected the model with lower AIC. Non-linear terms evaluated were polynomials, and natural splines quadratic and cubic. Convergence problems of some models were solved increasing the mathematical iterations, using optimizer (bobyqua optimizer) and centralized continuous variables if it was needed.

A residual analysis was made to all final models. Several covariates were missing in <5% of the participants and were imputed using fully conditional specification (Markov chain Monte Carlo method) with a maximum iteration number of five.

Supplemental Results

Non-returning participants:

From the 3,420 participants who were present at examination 1 but not at the follow-up examinations, 1,867 had died before the next follow-up visit. Of the 3,422 surviving participants, 1,553 did not return for the follow-up examinations. Survivors who did not return were older; more often women, hypertensive, current smoker, and diabetic; and had higher mean values for BMI, SBP, and HR. Among the echocardiographic parameters, the non-returning participants had larger LVM and left atrial (LA) diameter, larger chamber dimensions, higher relative wall thickness (RWT), smaller FS, higher A wave and DT, and lower E/A ratio. (Online Table 5)

Online Table 1. Baseline clinical and echocardiographic characteristics of the participants for the analysis of two left ventricular diastolic function parameters.

	WOMEN (n=882)	MEN (n=646)	p-value*
Clinical Features			
Age, years	73.63 (4.98)	73.64 (4.95)	0.990
BMI, kg/m²	27.27 (4.22)	27.02 (3.06)	0.201
SBP, mmHg	150.82 (20.86)	152.35 (20.13)	0.151
DBP, mmHg	85.50 (11.00)	85.81(10.98)	0.594
Blood pressure Lowering Medication, n (%)	414 (46.94)	338 (52.32)	0.038
Hypertension, n (%)	764 (86.6)	583 (90.3)	0.027
Heart Rate, beats/min	68.15 (9.46)	65.21 (10.64)	<0.001
Total Cholesterol, mmol/L	5.73 (1.04)	5.06 (1.04)	<0.001
HDL-cholesterol, mmol/L	1.64 (0.42)	1.34 (0.34)	<0.001
Lipid Lowering Medication, n (%)	232 (26.3)	236 (36.5)	<0.001
Current Smoker, n (%)	83 (9.4)	43 (6.7)	0.048

	WOMEN (n=882)	MEN (n=646)	p-value*
Clinical Features			
Prevalent CHD, n (%)	35 (3.97)	99 (15.3)	<0.001
Prevalent DM, n (%)	137 (15.5)	108 (16.7)	0.531
Echocardiography Features			
LVM index, g/m²	66.96 (14.80)	74.47(19.38)	<0.001
Left Atrium Diameter/BSA†, mm/m²	22.79 (2.93)	22.18 (2.83)	<0.001
LVEDD, mm	49.36 (4.29)	53.23 (5.07)	<0.001
LVESD, mm	28.31 (3.50)	31.35 (4.97)	<0.001
Relative Wall Thickness, cm	0.27 (0.05)	0.27 (0.05)	1.0
Fractional Shortening, %	43.14 (5.99)	41.92 (7.80)	<0.001

* p-value for comparison of different characteristics between men and women.
Values are mean (± standard deviation) or numbers (percentages).

BMI: Body mass index, BSA: Body surface area, CHD: coronary heart disease, DM: Type 2 diabetes mellitus, DBP: Diastolic blood pressure, LVEDD: Left ventricle end diastolic dimension, LVESD: Left ventricle end systolic dimension, LVM: Left ventricular mass, SBP: Systolic blood pressure.

Online Table 2. Left ventricular diastolic function parameters stratified by age and gender.

	55 – 64 years old			65 – 74 years old			>= 75 years old		
	Women	**Men**	**p-value***	**Women**	**Men**	**p-value***	**Women**	**Men**	**p-value***
E wave, cm/s	N:276 68 (13.08)	N:194 65.94 (12.41)	0.0867	N: 1253 67.37 (13.0)	N: 934 64.03 (12.8)	<0.001	N: 946 67.14 (13.19)	N: 693 64.6 (13.69)	<0.001
A wave, cm/s	N: 291 73.07 (13.27)	N: 201 68.83 (13.19)	<0.001	N: 1332 80.79 (17.04)	N: 960 73.53 (16.42)	<0.001	N:1013 89.86 (17.9)	N:711 82.2 (18.51)	<0.001
E/A ratio	N:274 0.947 (0.195)	N: 183 0.97 (0.20)	0.22	N: 1259 0.854 (0.175)	N: 875 0.88 (0.19)	0.0013	N:954 0.77 (0.17)	N: 658 0.80 (0.19)	<0.001
Deceleration Time, msec	N: 289 199 (33.25)	N: 207 201.86 (37.8)	0.365	N:1325 204.01 (33.83)	N:973 207.5 (38.48)	0.021	N: 1018 207.23 (39.24)	N: 714 213.67 (42.33)	0.0012
Septal e', cm/s	N/A†	N/A†	N/A†	N: 863 7.22 (1.78)	N:651 7.52 (1.67)	<0.001	N:981 6.5 (1.73)	N:713 6.99 (1.87)	<0.001
E/e' ratio	N/A†	N/A†	N/A†	N:799 10.06 (2.5)	N: 610 9.28 (2.41)	<0.001	N: 884 10.86 (2.73)	N:638 9.89 (2.64)	<0.001

Values are mean (± standard deviation).
* p-value for comparison of different values of left ventricular diastolic function parameters for women and men in each age group.
† N/A indicates that e' and E/e' ratio were not available at the indicated examination.

Online Table 3. Association of risk factors with longitudinal changes in left ventricular diastolic function parameters among women.

WOMEN	E wave	A wave	E/A	DT	e`septal	E/e`
	MIXED	MIXED	MIXED	MIXED	MIXED	MIXED
Agetime	†	†	†	†	‡	
BMI	‡	†				
SBP	†	†		‡		
DBP	†		†			
BP lowering medication						
Heart rate		†	†			
Total Cholesterol						
HDL Cholesterol					†	‡
Lipid low medication					‡	†
Current SMK				†		
LVM	‡				†	†
Prevalent CHD					‡	‡
Prevalent DM						
Ejection fraction						
Physical activity						
LAD						

*Age in this analysis is used as a time-varying covariate. †P< 0.0083 (significant at Bonferroni corrected P value); ‡P<0.05.

BMI: Body mass index, BP: blood pressure, CHD: Coronary heart disease, DBP: Diastolic blood pressure, DM: Diabetes Mellitus, HDL: High density lipoprotein, SBP: Systolic blood pressure.

Values are betas (95% confidence intervals). All presented betas (95% confidence intervals) are based on fully adjusted models. Sex-specific differences are highlighted in yellow in the table.

Online Table 4. Association of risk factors with longitudinal changes in left ventricular diastolic function parameters among men.

MEN	E wave	A wave	E/A	DT	e`septal	E/e`
	MIXED	MIXED	MIXED	MIXED	MIXED	MIXED
Agetime	†	†	†	†		
BMI		†	†	‡		†
SBP	†	†				†
DBP	†		‡			
BP lowering medication						
Heart rate	‡	†	†	‡		
Total Cholesterol						
HDL Cholesterol						
Lipid low medication						
Current SMK						
LVM	†				†	†
Prevalent CHD						†
Prevalent DM		‡			‡	‡
Ejection fraction			‡			
Physical activity						
LAD			‡			

*Age in this analysis is used as a time-varying covariate. †P< 0.0083 (significant at Bonferroni corrected P value); ‡P<0.05.

BMI: Body mass index, BP: blood pressure, CHD: Coronary heart disease, DBP: Diastolic blood pressure, DM: Diabetes Mellitus, HDL: High density lipoprotein, SBP: Systolic blood pressure.

Values are betas (95% confidence intervals). All presented betas (95% confidence intervals) are based on fully adjusted models. Sex-specific differences are highlighted in yellow in the table.

Online Table 5. Clinical and echocardiographic characteristics at the first examination for the individuals that participated only at the first examination and did not return for the two follow-up examinations.

	Participants (n=1,619)	Non-Returning Individuals (n=1,553)	p-value*
Clinical Features			
Age, years	67.45 (5.03)	71.47 (6.33)	<0.001
Female Sex, n (%)	931 (57.5)	1016 (65.4)	<0.001
BMI, kg/m²	27.37 (3.72)	27.88 (4.17)	<0.001
SBP, mmHg	144.70 (18.90)	150.16 (20.54)	<0.001
DBP, mmHg	80.85 (10.08)	80.18 (10.32)	0.065
Blood Pressure Lowering Medication, n (%)	531 (33.1)	708 (46.2)	<0.001
Hypertension, n (%)	1055 (65.9)	1140 (74.7)	<0.001
Heart Rate, beats/min	67.85 (10.27)	69.34 (10.93)	<0.001
Total Cholesterol, mmol/L	5.73 (0.98)	5.71 (0.96)	0.562
HDL-cholesterol, mmol/L	1.47 (0.39)	1.49 (0.40)	0.154
Lipid Lowering Medication, n (%)	343 (21.4)	355 (23.2)	0.568
Current Smoker, n (%)	166 (10.5)	207 (13.6)	0.363
Prevalent CHD, n (%)	91 (5.6)	106 (6.8)	0.728
Prevalent DM, n (%)	160 (9.9)	201 (12.9)	0.376
Echocardiography Features†			
LVM index, g/m²	72.29 (17.33)	74.35 (18.19)	0.013
Left Atrium Diameter/BSA‡, mm/m²	21.13 (2.61)	21.56 (2.99)	<0.001
LVEDD, mm	51.08 (5.03)	50.91 (5.28)	0.353
LVESD, mm	30.71 (4.76)	31.11 (5.30)	0.025
Relative Wall Thickness, cm	0.29 (0.05)	0.30 (0.06)	<0.001
Fractional Shortening, %	38.52 (14.54)	36.50 (16.26)	<0.001
E wave, cm/s	65.53 (14.56)	64.97 (15.74)	0.305
A wave, cm/s	73.26 (15.58)	77.32 (16.73)	<0.001
Deceleration Time, msec	207.86 (40.43)	214.15 (46.86)	<0.001
E/A ratio	0.93 (0.23)	0.88 (0.36)	<0.001

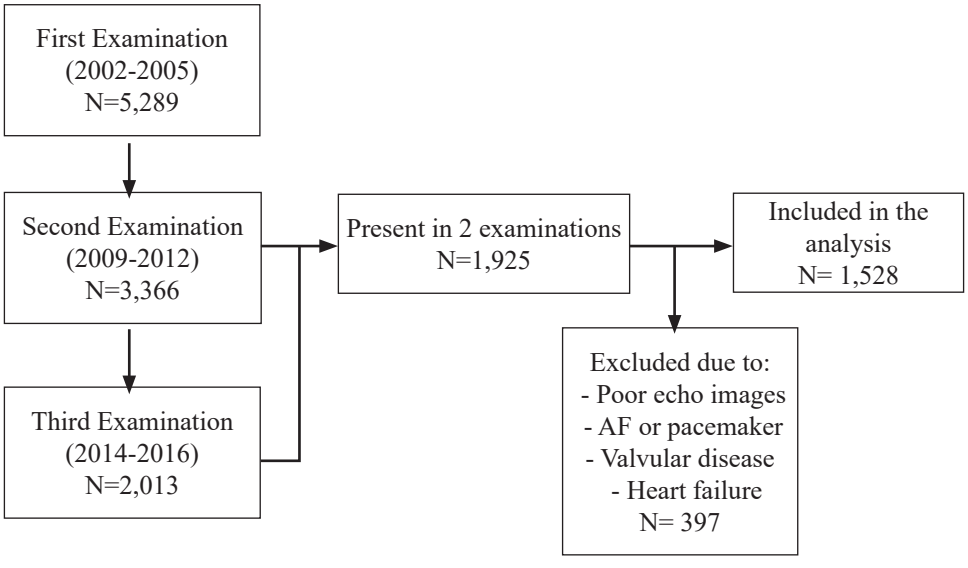
*p-value for comparison of different characteristics between participants and non-returning individuals.

† In the first evaluation measurements of Echo TDI were not available.

Values are mean (± standard deviation) or numbers (percentages).

BMI: Body mass index, BSA: Body surface area, CHD: coronary heart disease, DM: Type 2 diabetes mellitus, DBP: Diastolic blood pressure, LVEDD: Left ventricle end diastolic dimension, LVESD: Left ventricle end systolic dimension, LVM: Left ventricular mass, SBP: Systolic blood pressure.

Online Figure 1. Flow chart for the participants included in the analysis of two left ventricular diastolic function parameters measured two times.



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CHAPTER 2.3

Impact of cumulative systolic blood pressure and serious adverse events on efficacy of intensive blood pressure treatment: a randomized clinical trial.

Selected to participate in the final round of SPRINT challenge organized by The New England Journal of Medicine, 2017.

First place in crowding voting phase of SPRINT challenge NEJM, 2017

Accepted oral presentation, advances in science, European Society of Cardiology, Congress 2017.

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Abstract published in: Circulation, Vol. 138, Suppl_1: A15083, November 2018.

Full text published in: Journal of Hypertension, Vol. 37 (5), 1058-1069, May 2019.

Editorial comment published in: Journal of Hypertension, Vol. 37 (5), 902-904, 2019

ABSTRACT

BACKGROUND: Intensive blood pressure lowering is increasingly gaining attention. In addition to higher baseline blood pressure, cumulative systolic blood pressure (SBP), visit-to-visit variability and treatment-induced serious adverse events (SAEs) could impact treatment efficacy over time. Our aim was to assess the impact of cumulative SBP and SAEs on intensive hypertension treatment efficacy in the systolic Blood Pressure Intervention Trial (SPRINT) population during follow-up.

METHODS: Secondary analysis of The SPRINT study; a randomized, controlled, open label trial including 102 clinical sites in the United States. We included 9068 SPRINT participants with 128139 repeated SBP measurements. Participants were randomly assigned to intensive (target SBP <120 mmHg) vs standard treatment (target SBP between 130-139 mmHg). We used cumulative joint models (cJM) for longitudinal and survival data analysis. Primary outcome was a composite outcome of myocardial infarction, other acute coronary syndromes, acute decompensated heart failure, stroke, and cardiovascular mortality.

RESULTS: Although intensive treatment decreased the risk for the primary SPRINT outcome at the start of follow-up, its effect lost significance after 3.4 years of follow-up in the total SPRINT population and after 1.3, 1.3, 1.1, 1.8, 2.1, 1.8, and 3.4 years among participants with prevalent chronic kidney disease, prevalent cardiovascular disease, women, black individuals, subjects <75 years, those with baseline SBP >132 mmHg, and individuals who suffered SAEs during follow-up respectively.

CONCLUSIONS: The initial beneficial impact of intensive hypertension treatment might be offset by cumulative SBP and development of SAEs during follow-up.

CLINICAL TRIAL REGISTRATION: URL: https://www.clinicaltrials.gov. Unique identifier: NCT01206062.

CONDENSED ABSTRACT: Based on cumulative joint model analysis, intensive treatment decreased the risk for the primary SPRINT outcome at the start of

follow-up. However, its effect lost significance after 3.4 years of follow-up in the total SPRINT population and after 1.3, 1.3, 1.1, 1.8, 2.1, 1.8, and 3.4 years among participants with prevalent chronic kidney disease, prevalent cardiovascular disease, women, black individuals, subjects <75 years, those with baseline SBP >132 mmHg, and individuals who suffered SAEs during follow-up respectively. The initial beneficial impact of intensive hypertension treatment might be offset by cumulative SBP and development of SAEs during follow-up.

KEYWORDS: Adverse effects, Cumulative Joint Model, Intensive treatment, Randomized controlled trial, Systolic blood pressure, Treatment efficacy.

INTRODUCTION

High blood pressure is a major modifiable risk factor for cardiovascular disease (CVD)[1, 2]. In addition to higher baseline blood pressure, visit-to-visit variability and cumulative exposure to blood pressure have been linked to higher risk for CVD and kidney dysfunction[3-5]. Intra-individual blood pressure fluctuations are not random and tend to persist within individuals[6, 7]. Therefore, the conventional approach of correlating baseline blood pressure with outcomes of interest in clinical trials might lead to biased estimates regarding treatment efficacy.

Treatment of hypertension and lowering blood pressure has been consistently associated with beneficial clinical outcomes in observational studies and randomized clinical trials[8]. Since the epidemiological associations of blood pressure with cardiovascular risk do not indicate a clear lower-bound threshold[9], lowering the blood pressure to the lowest tolerable levels is deemed to yield the greatest clinical benefit[10, 11]. However, intensive blood pressure lowering has adverse effects that could impact the efficacy of this intervention over time[12]. Recent evidence from the SPRINT trial showed that intensive lowering of blood pressure significantly reduced major vascular events[13]. Nevertheless, several serious adverse events (SAEs), such as hypotension, acute kidney injury and electrolytes abnormalities, occurred more often in the intensive treatment compared to the standard treatment group. It, therefore, remains unclear whether benefits from intensive lowering of blood pressure outweigh the risk for adverse events over the course of treatment. Taking into account the cumulative effect of the SBP, its intra-individual variability and the adverse effects produced during the follow-up, we asked the question: Do the beneficial effects of intensive SBP reduction remain in the long term in SPRINT total population and in each subgroups under analysis?.

Using the SPRINT database, we aimed to assess the impact of cumulative exposure to blood pressure on the beneficial effects of intensive hypertension treatment. We further sought to evaluate the impact of SAEs on the efficacy of intensive treatment during follow-up.

METHODS

Original SPRINT Trial

The SPRINT trial included 9361 hypertensive participants with systolic blood pressure (SBP) between 130-180 mmHg, older than 50 years, with increased cardiovascular risk. Exclusion criteria were diabetes mellitus, stroke, advanced chronic kidney disease (CKD), proteinuria >1 g/d, polycystic kidney disease, congestive heart failure (HF), dementia, or residence in a nursing home. Participants were randomly assigned to intensive (target SBP <120 mmHg) versus standard treatment (target SBP between 130-139 mmHg) and were evaluated monthly during the first trimester of follow-up and every 3 months afterwards. The trial stopped at 3.26 years median follow-up (range 0 to 4.5 years) based on recommendation from the data safety monitoring board. Primary outcome was a composite of myocardial infarction, other acute coronary syndromes, acute decompensated HF, stroke, and cardiovascular mortality. SAEs were the events meeting any of the following criteria: fatal or life-threatening event resulting in significant or persistent disability, required or prolonged hospitalization, representing significant hazards or harm to research participants potentially requiring medical or surgical intervention[13].

Our Secondary Analysis of SPRINT Trial

For this secondary analysis, we used the original SPRINT database available by data request #4536 to BioLINCC repository (National Heart, Lung and Blood Institute) under the SPRINT data analysis challenge initiative, organized by The New England Journal of Medicine. Our research protocol was approved by the Ethical Committee of Universidad Industrial de Santander, Bucaramanga, Colombia. Subjects with missing data on covariates or without repeated SBP measurements and observations occurring after the primary event were removed. After exclusion of 293 participants (26 primary outcomes), the current analyses included 9068 participants (97% of the original SPRINT participants) with 128139 SBP measurements and 536 primary outcomes (95.4% of the original SPRINT outcomes) (Figure 1).

Statistical analysis

Two researchers independently built the long format database for the analyses to ensure no data management inconsistences. Firstly, we focused on analyzing the SBP longitudinal evolutions. To account for the correlations among the repeated measurements of each patient, we used linear mixed effects models (LMM). Initial descriptive analysis showed that patients experienced an immediate SBP drop after initiation of treatment (Figure 2). To account for this feature in both the fixed and random-effects parts of the LMM, we used natural cubic splines with internal knots placed at 0.25, 0.5 and 1.4 years, and boundary knots (in this case the upper knot) not to the maximum (i.e., the default) but to the 95% percentile of the time variable (0, 3.5 year) to capture the time evolutions. We used a diagonal covariance matrix (pdDiag) in the random argument. The treatments effect was included in the fixed-effects part both as main effects and interacting with the nonlinear time effect. Second, for the primary SPRINT outcome a Cox model was used in which again treatment was included as an explanatory variable. Finally, to explicitly capture the association between the serial SBP measurements of each patient and the hazard of the primary outcome, we utilized the framework of joint models for longitudinal and survival outcomes[14-17]. This framework combines the two aforementioned mixed effects and Cox models. In the specific joint model we used (cumulative joint model - cJM), we accounted for the cumulative exposure of SBP (that is the whole history of SBP values of each patient) to the hazard of the primary endpoint. Regarding treatment differences, the major advance of joint models versus the traditional Cox model is that they allow to disentangle the total treatment effect into two parts (Figure 3); namely a direct effect of treatment to the hazard of the endpoint and an indirect effect of treatment via SBP. We also derived the total treatment effect from the cJM that accounts for differences in SBP values over time, and associated 95% confidence intervals using Bootstrapping. (See Supplemental Appendix for additional information about cJM)

Additionally, we examined the distribution of different types of SAEs in the total population and in each subgroup. We further evaluated the risk of developing SAEs related to the intervention type (intensive versus standard treatment) using Cox proportional hazards analyses. We then introduced an interaction term in the cJM for occurrence of SAEs during follow-up and stratified the analyses accordingly. Similar to the original SPRINT report, we performed subgroup analyses among CKD/non-CKD, female/male, black/non-black race, age <75/≥75, CVD/non-CVD, and baseline SBP categories (≤132 mmHg, 133-144 mmHg, and ≥145 mmHg). All analyses were performed using JM package[15] of R software (R 3.4.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Table 1 presents characteristics of the 9068 included participants. Similar to the original SPRINT report, the intensive and standard treatment groups are balanced in all variables.

Figure 2 shows the average SBP changes during follow-up in the intensive and standard treatment groups (these averages were estimated through the fixed-effects part of the LMM). Figure 4 shows the SBP variability within each individual for several randomly selected participants (this variability was estimated in all participants through the random-effects part of the LMM). The plot indicates a large variability of SBP within individuals. Most of the decline in SBP occurred during the first three months of follow-up. In the LMM, mean SBP at baseline was 139.7 mmHg in both groups. Intensive treatment significantly reduced SBP by an average 12.73 mmHg during follow-up. This corresponds to the overall difference in average SBP during follow-up between the intensive and standard treatment groups depicted in Figure 2. HR for intensive treatment in the overall population, using traditional Cox model, was similar to the original SPRINT report (HR; 95%CI: 0.75; 0.63, 0.89). The cJM approach HR (95%CI) was 0.60 (0.50, 0.72) at the start of follow-up. However, the effect significance was lost after 3.36 years (Figure 5).

In all subgroups, HRs for intensive treatment using traditional Cox model were similar to the original SPRINT report. Using the cJM approach, intensive treatment decreased the risk for the primary outcome among all subgroups at the start of follow-up. However, the effect lost its significance after 1.3 and 3.4 years among subjects with and without baseline CKD, after 1.1 and 3.5 years among women and men, after 1.8 and 3.1 years among black and non-black individuals, after 2.1 and 3.4 years among individuals <75 years and >=75 years, after 1.3 and 3.4 years among subject with and without prevalent CVD, after 2.5, 2.0 and 1.8 years for individuals with baseline SBP <=132 mmHg, between 133-144 mmHg, and >=145 mmHg respectively (Figure 6 A-L). Appendix table S1 presents the HRs (95% CIs) at the start and end of follow-up for all subgroups.

SAEs occurred in 96.3% (n=516) of participants who suffered the primary outcome. Appendix table S2, shows distribution of SAEs by subgroup. Using Cox proportional hazards analyses (HR; 95%CI), hypotension (1.71; 1.26, 2.33), electrolyte abnormalities (1.38; 1.07, 1.79), and acute renal failure (1.68; 1.33, 2.12) were significantly associated with intensive treatment (Table 2). In the cJM, the interaction term for having experienced SAEs during follow-up in the overall population was significant (P for interaction SAEs*treatment <0.0001). Therefore,

we stratified the cJM analyses based on occurrence of SAEs during follow-up. Cox analyses HRs (95%CI) for intensive treatment in groups with and without SAEs were 0.74 (0.62, 0.88) and 0.25 (0.084, 0.75) respectively. Using the cJM approach, the HRs (95%CI) at the start of follow-up were 0.60 (0.50, 0.72) and 0.19 (0.06, 0.63) for the groups with and without SAEs respectively (Table S1). The effect lost significance after 3.4 years for subjects with SAEs but remained significant until 4.2 years of follow-up for the non-SAEs group. The wider 95%CI for the non-SAEs group reflects the small number of primary outcomes in this group (n=20; 4% of all primary outcomes) (Figure 6 M, N). Finally, we evaluated the differential effect of SAEs in the SPRINT primary outcome among interventions groups using Cox proportional analysis. We found significantly three time larger effects of SAEs on the hazard ratio for primary outcome, in the intensive treatment group (HR: 96.95; $P < 0.000$) compared with standard treatment (HR: 33.42; $P< 0.000$) in the overall SPRINT population.

DISCUSSION

Our secondary analysis of SPRINT trial confirmed that intensive hypertension treatment lowered the risk for the primary outcome at start of follow-up. However, the initial beneficial effect was lost during follow-up in the overall population and particularly among participants with prevalent CKD or CVD, women, black individuals, younger subjects, and those with SBP above 132 mmHg at baseline. The beneficial effect of intensive treatment was also lost earlier among patients who suffered SAEs during follow-up.

Conventionally, trials correlate the baseline blood pressure values with outcomes of interest. The original SPRINT analysis showed a 25% reduction in the primary outcome for intensive treatment, using the traditional Cox approach assuming that the benefits remain constant over time. However, besides higher blood pressure at baseline, cumulative exposure to blood pressure and its variability, are important risk factors for CVD and kidney dysfunction[4-6]. Our analyses simultaneously took into account the dependency and association between repeated SBP measurements and time-to-event and allowed for evaluation of both direct and indirect (i.e. through SBP) effects of the intensive treatment[14-17]. When cumulative effect of SBP and its variability, both within individuals and between treatment groups, was taken into account, the initial beneficial effect of intensive treatment was lost during follow-up. Importantly, recent secondary analyses of ONTARGET and TRASCEND trials showed a higher predictive value for a composite mean SBP over time compared with baseline or event-preceding or time-updated SBP[18], which substantiates our

approach. Based on experimental studies, high blood pressure variability induces a chronic inflammatory state through activation of the myocardial angiotensin-converting enzyme, increasing the expression of monocyte-protein-1 and transforming growth factor-B, resulting in ventricular hypertrophy, remodeling and dysfunction, perivascular fibrosis, endothelial injury and kidney dysfunction[19-23].

Three recent studies investigating the association of visit-to-visit blood pressure variability with primary SPRINT outcome and adverse events have led to conflicting results. Chang et al showed no association between blood pressure variability with primary SPRINT outcome but a significant association with all-cause mortality[24]. This study, however, included only the SBP measurements between 3-18 months of follow-up and discarded about 42% (n=238) of the primary SPRINT outcomes. Moreover, they adjusted for multiple covariates disregarding the previous treatment randomization. Goyal et al showed SBP variability to be independently associated with higher risk of hyponatremia among SPRINT participants[25]. In another post-hoc analysis among a subset of SPRINT participants with baseline CKD, diastolic blood pressure variability was associated with the primary outcome and with major SAEs[26].

The beneficial effect of intensive treatment was lost earlier among specific subgroups in our analyses; including CKD participants, women, and individuals of black ethnicity. Previous studies have observed larger SBP variability among these groups, linking it to a higher vascular risk among these individuals[23, 27]. Their larger SBP variability might explain earlier loss of beneficial effect of intensive treatment among these individuals. Compared to older subjects, individuals younger than 75 years lost the beneficial effect of intensive treatment earlier. While SBP variability increases with age, younger individuals have shown a greater susceptibility to target organ damage resulting from SBP variability[27]. Moreover, older patients might respond better to medications such as diuretics due to their beneficial impact on outcomes such as HF which is one component of the primary SPRINT outcome[28]. These factors might explain earlier loss of beneficial impact of intensive treatment among younger individuals in our study. We also observed that individuals with SBP >132 mmHg at baseline and during follow-up lost the beneficial impact of intensive treatment earlier compared to those with SBP<=132. This could be attributed to a higher SBP variability among individuals with SBP >132 mmHg due to larger fluctuations in the number or dose of prescribed antihypertensive medications in this group.

Intensive blood pressure lowering could lead to adverse events altering the efficacy of this intervention during follow-up. Our study showed less benefit for intensive

treatment among individuals who experienced SAEs during follow-up (Figure 6 M, N). Although the proportion of participants who suffered SAEs was similar between the intensive and standard treatment groups, type of adverse event was different. More severe adverse events including hypotension, electrolyte abnormalities, and acute kidney injury occurred more often in the intensive treatment group. Besides cumulative SBP and its variability, development of SAEs could partly explain loss of initial beneficial effect for intensive treatment over time. A secondary analysis of SPRINT trial among participants with normal renal function at baseline showed a 1.2 ratio for developing CKD per preventing 1 cardiovascular event[29]. The risk for mortality and CVD among patients with renal dysfunction is between 1.2-1.8 and 1.9-2.9 respectively[30]. Projecting the SPRINT eligibility criteria to the 1999-2006 National Health and Nutrition Examination Survey showed that intensive treatment prevents 107500 deaths per-year but increases the number of patients with SAEs to 222600 per-year[12]. Notably, SAEs preceded the primary outcome in the majority of SPRINT participants (96.3%). In the Intensive treatment group, SAEs were associated with SPRINT primary outcome three times more than the SAEs in the standard group. If SAEs increase the risk of primary outcome, the harms of intensive hypertension treatment might offset its potential benefits.

New guidelines for management of blood pressure, re-define the therapeutic target as BP <130/80 mmHg[31, 32]. For primary prevention, the guidelines recommend pharmacology treatment among individuals with BP >130/80 mmHg and cardiovascular risk >10% or those with cardiovascular risk <10% but BP >140/90 mmHg. In secondary prevention settings, pharmacology treatment is recommended for BP >130/80. However, our results in the subgroup of SPRINT participants with CVD history showed earlier loss of beneficial impact of intensive SBP treatment over time than for non-CVD participants.

Despite the observed increasing tendency in the HRs over time, as the SPRINT trial terminated after median 3.26 years of follow-up (Range 0 to 4.5 years), our findings are only applicable to this time-window. 96.3% of patients who developed a primary outcome suffered SAEs during follow-up. This led to small number of events and limited power for the analyses among participants without SAEs.

Major strength of our study is the use of a robust statistical model which allows us to maintain the initial SPRINT randomization in our analyses. Additionally, our approach allows for evaluation of the cumulative impact of SBP and its variability (both intra-individual and between groups) as well as SAEs on the primary SPRINT outcome, taking into account that HRs may change over time[33]. An additional benefit of using joint model analysis is that post-randomization BP measurements

are treated as an outcome (and not as a covariate), the joint likelihood of the BP measurements and the time to the primary endpoint are also completely specified, thus providing valid estimates of the treatment effect. During development of the statistical model and for construction of different SBP trajectories over time, we specifically took into account the initial decrease in SBP at the beginning of follow-up. As model specification and goodness of fit are fundamental for the validity of our results, supplemental statistical material details all the steps we followed for development of our statistical models.

Finally, we are aware that our results may be considered controversial, however, they are in line with what was originally published by the SPRINT group (Figure 4 original publication NEJM)[13], which showed that intensive treatment did not significantly reduce cardiovascular risk in patients with CKD, younger participants, females, black individuals, CVD and those with baseline SBP> 132 mmHg. These groups are the same ones in which we have found that the protective benefit of intensive treatment is lost early during follow-up. Thus, loss of beneficial effect occurred earlier in those who did not significantly benefit from intensive treatment in the original SPRINT trial. These findings further increase confidence in the validity of our results.

CONCLUSION

Intensive SBP treatment lowered the risk for the primary SPRINT outcome at the start of follow-up. However, the initial beneficial effect was lost during follow-up in the overall population and particularly among participants with prevalent CKD or CVD, women, black individuals, younger participants, those with baseline SBP >132 mmHg, and patients who suffered SAEs.

Our results call for caution regarding universal recommendations for intensive blood pressure treatment, particularly among specific subgroups. Besides potential adverse effects from intensive treatment, the impact of cumulative SBP as well as intra-individual SBP variability should not be dismissed. As the tenet of medicine “Primum non nocere” must prevail, longer-term clinical trials with a particular focus on sustained beneficial effects of intensive interventions over time and on patient safety are needed. Cumulative joint model (cJM) analysis is a novel and not frequently considered approach for assessment of clinical trial data. This method adds a time-varying perspective which approaches the conditions encountered in daily clinical practice.

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Table 1. Baseline Characteristic of the Study Participants

Characteristic*	Intensive Treatment (N= 4552)	Standard Treatment (N= 4516)	p-value
Criterion for increased cardiovascular risk, n (%)†			
Age ≥75 years	1276 (28.03)	1258 (27.86)	0.853
Chronic kidney disease‡	1296 (28.47)	1262 (27.95)	0.578
Cardiovascular disease	921 (20.23)	905 (20.04)	0.818
Clinical	762 (16.74)	757 (16.76)	0.977
Subclinical	245 (5.38)	237 (5.25)	0.776
Framingham 10-year CVD risk score ≥15%	2800 (61.51)	2782 (61.6)	0.928
Female sex, n (%)	1625 (35.70)	1582 (35.03)	0.506
Age, years			
Overall	67.9±9.4	67.8±9.4	0.756
Among those ≥75 years of age	79.8±3.8	79.8±3.9	0.846
Race or ethnic group, n (%)§			
Non-Hispanic black	1338 (29.39)	1371 (30.36)	0.228
Hispanic	492 (10.81)	470 (10.41)	
Non-Hispanic white	2626 (57.69)	2603 (57.64)	
Other	96 (2.11)	72 (1.59)	
Black race‖	1413 (31.04)	1438 (31.84)	0.411
Baseline blood pressure, mmHg			
Systolic	139.67±15.8	139.67±15.4	0.993
Diastolic	78.2±11.9	78.1±12.0	0.519
Distribution of systolic blood pressure, n (%)			
≤132 mmHg	1543 (33.90)	1490 (33.00)	0.345
>132 mmHg	1451 (31.88)	1504 (33.30)	
≥145 mmHg	1558 (34.23)	1522 (33.70)	
Serum creatinine, mg/dl	1.07±0.34	1.07±0.33	0.869
Estimated GFR, ml/min/1.73m^2			
Among all participants	71.81±20.6	71.83±20.5	0.973

Characteristic*	Intensive Treatment (N= 4552)	Standard Treatment (N= 4516)	p-value
Among those with estimated GFR ≥60	81.4±15.5	81.1±15.5	0.522
Among those with estimated GFR <60	47.9±9.4	47.9±9.5	0.907
Ratio of urinary albumin (mg) to creatinine (g)	43.0±174.5	41.2±154.4	0.612
Fasting total cholesterol, mg/dl	190.1±41.5	190.2±41.1	0.971
Fasting HDL cholesterol, mg/dl	52.92±14.4	52.76±14.5	0.593
Fasting total triglycerides, mg/dl	125.1±86.4	127.2±94.2	0.262
Fasting plasma glucose, mg/dl	98.9±13.8	98.8±13.3	0.797
Statin use, n (%)	1947 (42.77)	2019 (44.71)	0.063
Aspirin use, n (%)	2348 (51.66)	2278 (50.52)	0.278
Smoking status, n (%)			
Never smoked	1994 (43.80)	1990 (44.07)	0.670
Former smoker	1934 (42.49)	1936 (42.87)	
Current smoker	622 (13.66)	586 (12.98)	
Missing data	2 (0.04)	4 (0.09)	
Framingham 10 year CVD risk score, %	20.06±10.9	20.1±10.8	0.789
Body-mass index¶	29.92±5.8	29.81±5.7	0.3732
Antihypertensive agents, n (%)	1.85±1.04	1.83±1.04	0.379
Not using antihypertensive agents, n (%)	419 (9.20)	437 (9.68)	0.442

* Plus–minus values are means ±SD. There were no significant differences (P<0.05) between the two groups. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. To convert the values for glucose to millimoles per liter, multiply by 0.05551. CVD denotes cardiovascular disease, GFR glomerular filtration rate, HDL high-density lipoprotein, and n or N numbers.

† Increased cardiovascular risk was one of the inclusion criteria.

‡ Chronic kidney disease was defined as an estimated glomerular filtration rate of less than 60 ml per minute per 1.73 m2 of body surface area.

§ Race and ethnic group were self-reported.

‖ Black race includes Hispanic black and black as part of a multiracial identification.

¶ The body-mass index is the weight in kilograms divided by the square of the height in meters

Table 2. Association of Intensive Treatment with Serious Adverse Events During Follow-up

Characteristic	Intensive Treatment (N= 4552)	Standard Treatment (N= 4516)	HR (CI 95%)	p-value
All serious adverse events*	1748 (38.40)	1676 (37.11)	1.04 (0.98-1.12)	0.210
Specific conditions of interest				
Hypotension	110 (2.42)	64 (1.42)	1.71 (1.26-2.33)	0.001
Syncope	104 (2.28)	80 (1.77)	1.29 (0.96-1.73)	0.087
Bradycardia	86 (1.89)	70 (1.55)	1.22 (0.89-1.67)	0.222
Electrolyte abnormality	142 (3.12)	102 (2.26)	1.38 (1.07-1.79)	0.012
Injurious fall†	104 (2.29)	105 (2.33)	0.98 (0.75-1.29)	0.887
Acute kidney injury or acute renal failure‡	192 (4.22)	114 (2.52)	1.68 (1.33-2.12)	0.000

CI denotes confidence interval, HR hazard ratio, and N numbers.

Serious adverse events include conditions of interest classified as possibly or definitely related to the intervention by the SPRINT investigators. * Defined as an event that was fatal or life threatening, resulting in significant or persistent disability, requiring or prolonging a hospitalization, or was an important medical event that the investigator judged to be a significant hazard or harm to the participant that may have required medical or surgical intervention to prevent one of the other events listed above; † An Injurious fall was defined as a fall that resulted in evaluation in an emergency department or resulted in hospitalization; ‡ Acute Kidney Injury and Acute renal failure were coded if the diagnosis was listed in the hospital discharge summary and was felt to be one of the top 3 reasons for admission or continued hospitalization. A few cases of AKI were noted in an emergency department if the participant presented for one of the other conditions of interest.

Figure 1. Flow chart of the original SPRINT trial participants and participants in our secondary analysis of SPRINT trial

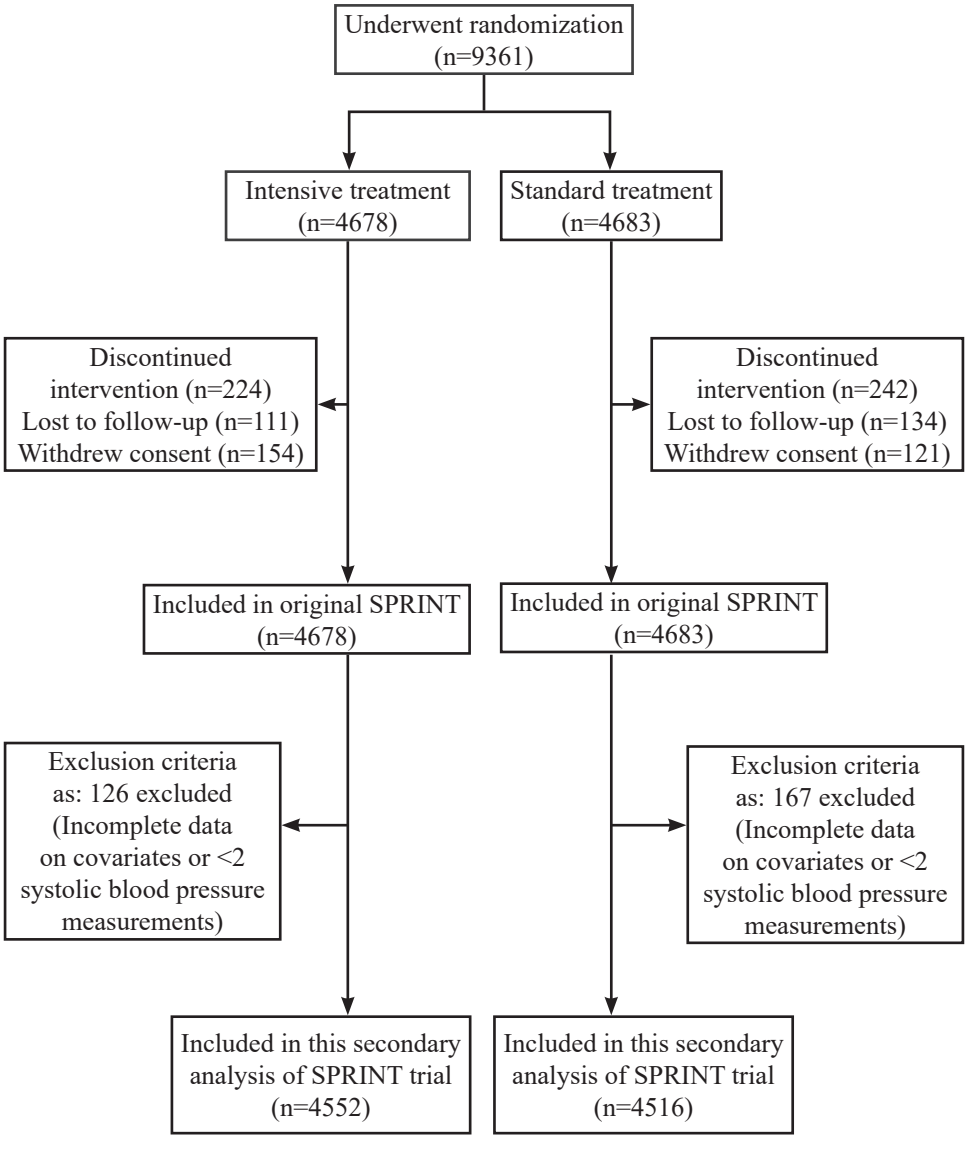


Figure 2. Mean systolic blood pressure trajectories for the intensive treatment and standard treatment groups in SPRINT trial

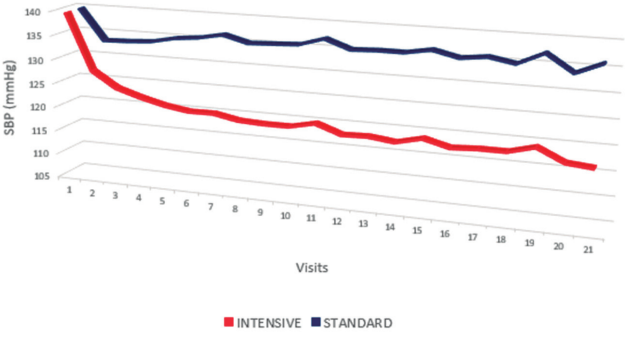
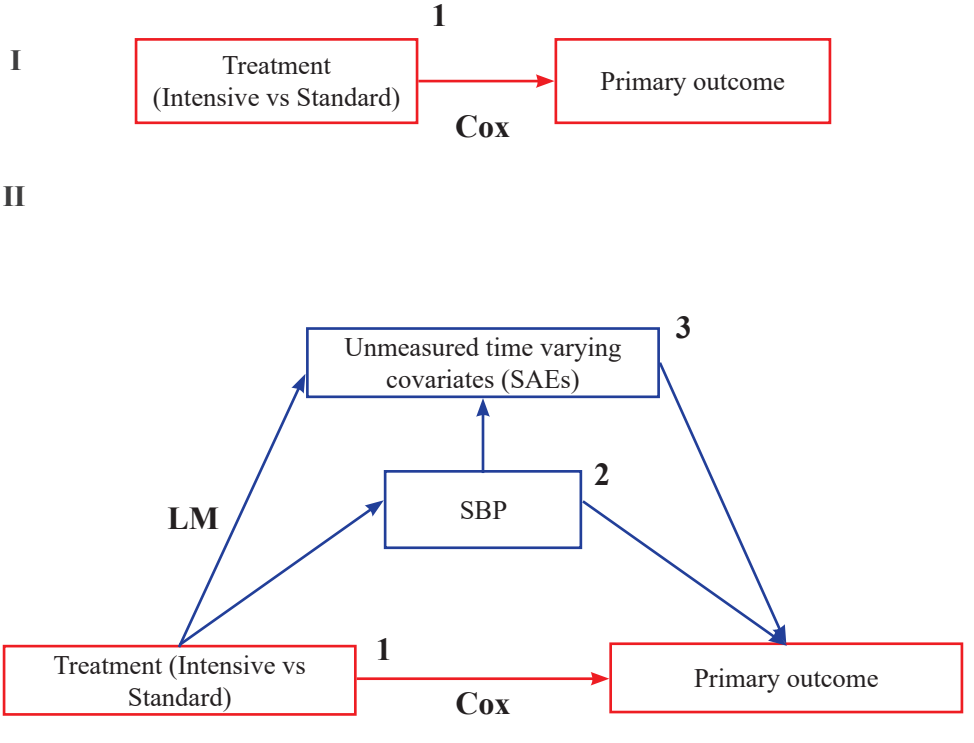


Figure 3. Comparison between Traditional Cox Proportional Hazards and Cumulative Joint Model Approaches in the Total SPRINT Population



Cox denotes Cox proportional hazard model, LMM linear mixed effects model, SAEs serious adverse events, SBP systolic blood pressure.

I. Traditional Cox model analysis
II. Joint model for longitudinal and time-to-event-data
1. Baseline characteristics between intervention groups are balanced by randomization
2. Changes in SBP over time between individuals by groups (fixed part of LMM) and changes in SBP over time within individuals by groups (random part of LMM)
3. All (including unmeasured) time varying covariates (such as SAEs) (random part of LMM)

Figure 4. Intra-individual SBP variability during follow-up for several randomly selected SPRINT participants

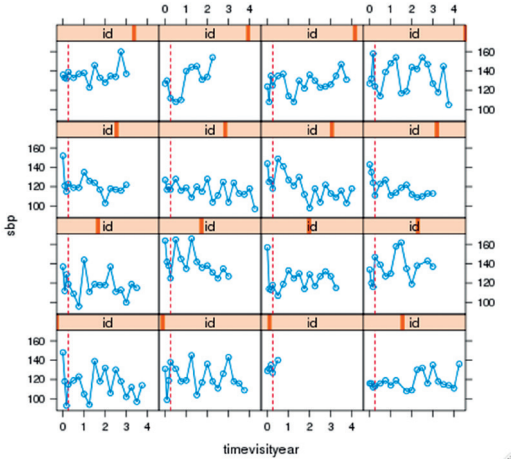
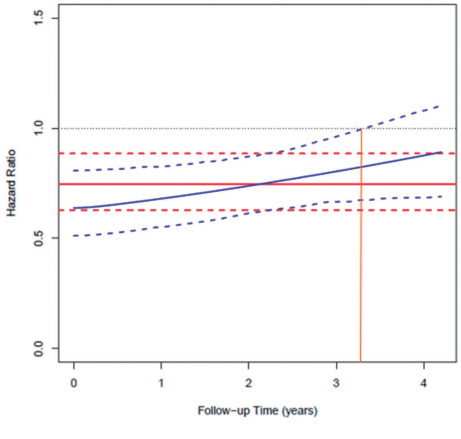
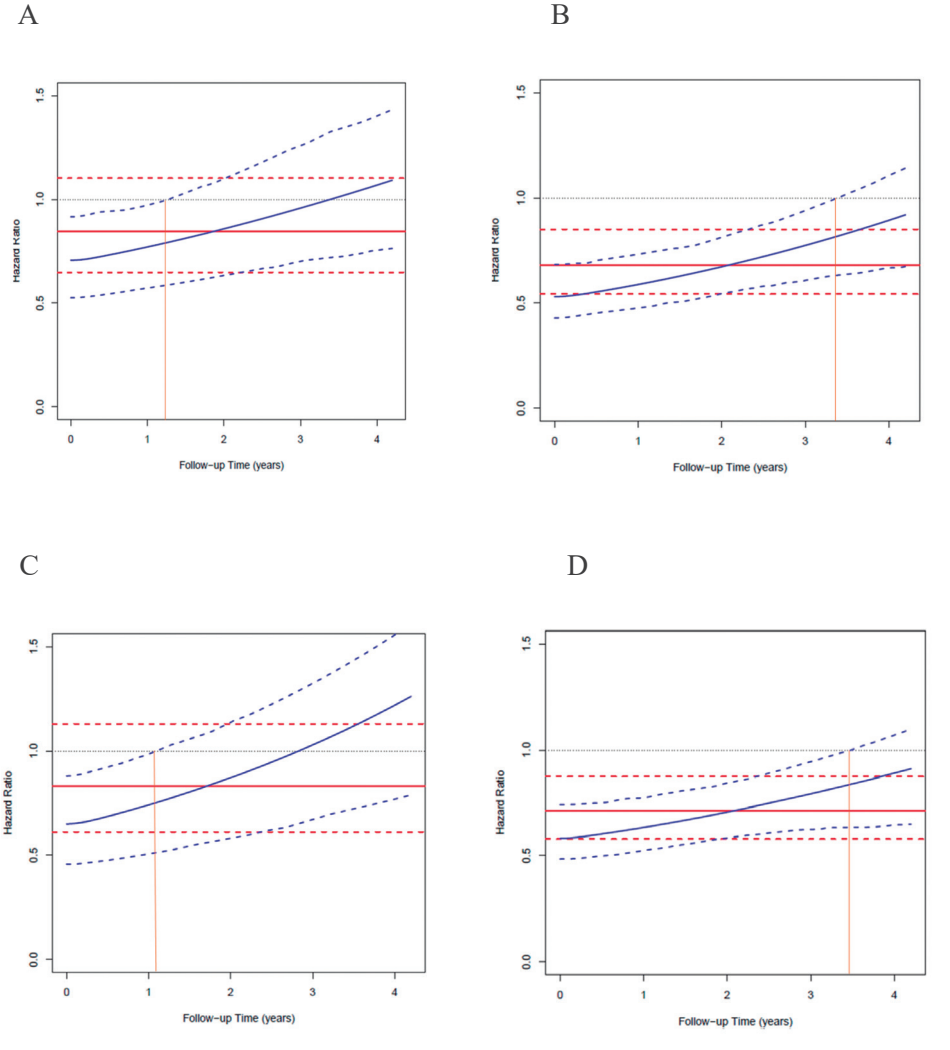


Figure 5. Dynamic changes in hazard ratio for primary SPRINT outcome over time in total population

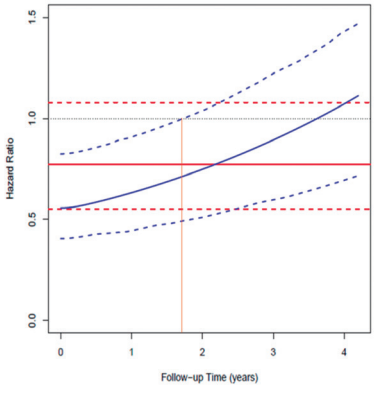


Hazard ratio and 95% confidence interval for intensive SBP treatment based on traditional Cox proportional hazard approach (red lines) and cumulative joint model approach (blue lines). Orange vertical line denotes the time point at which the statistical significance of the effect estimate is lost.

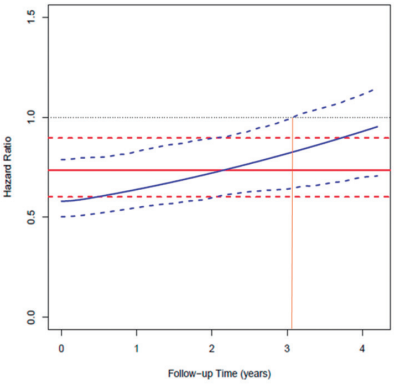
Figure 6. Hazard ratio for primary SPRINT outcome over time based on Traditional Cox Proportional Hazards and Cumulative Joint Model Approaches among SPRINT Subgroups



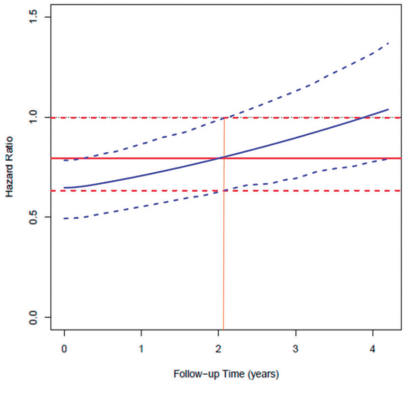
E


Hazard Ratio
0.0
0.5
1.0
1.5
0
1
2
3
4
Follow-up Time (years)

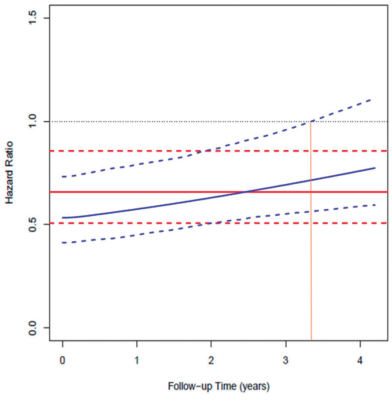
F


Hazard Ratio
0.0
0.5
1.0
1.5
0
1
2
3
4
Follow-up Time (years)

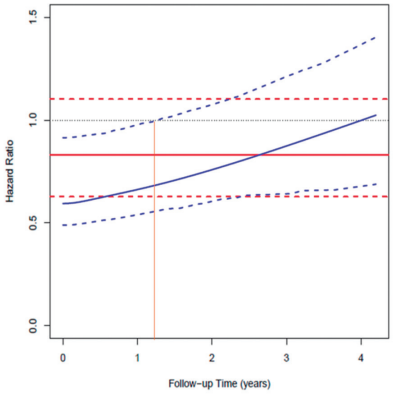
G


Hazard Ratio
0.0
0.5
1.0
1.5
0
1
2
3
4
Follow-up Time (years)

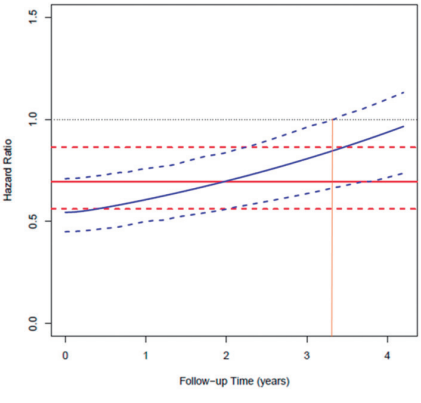
H

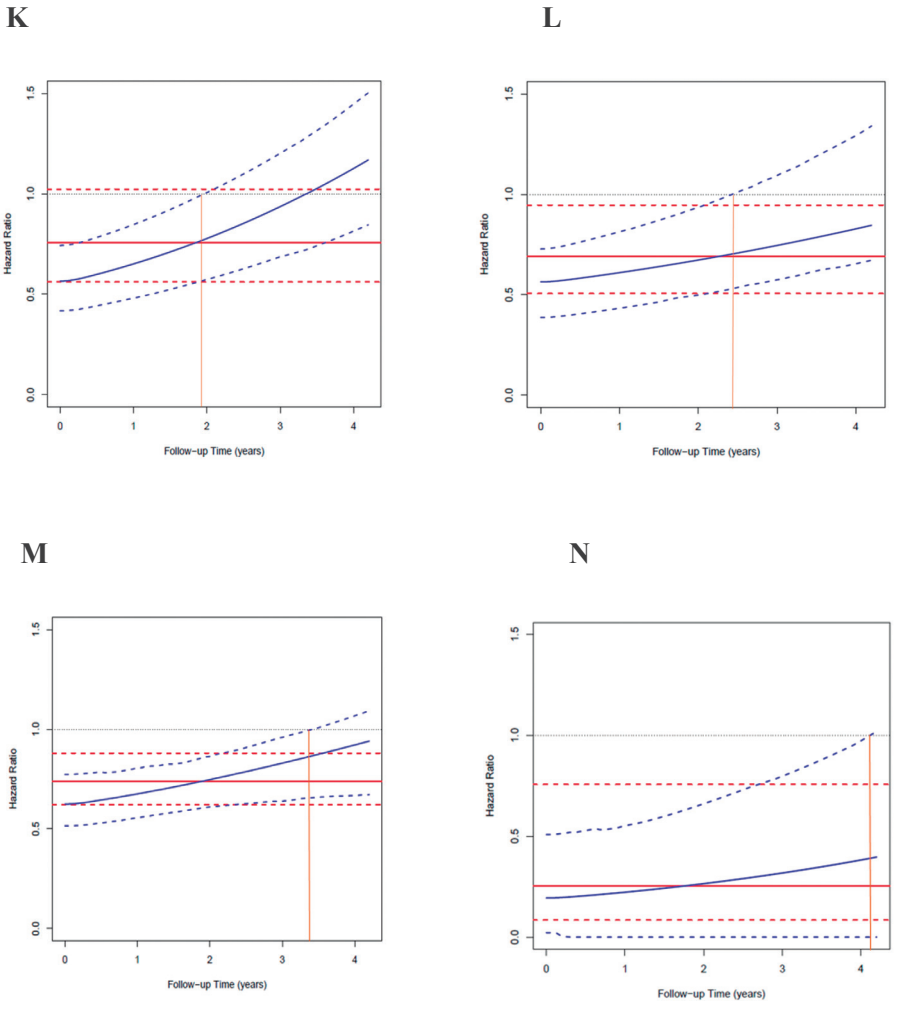

Hazard Ratio
0.0
0.5
1.0
1.5
0
1
2
3
4
Follow-up Time (years)

I


Hazard Ratio
0.0
0.5
1.0
1.5
0
1
2
3
4
Follow-up Time (years)

J


Hazard Ratio
0.0
0.5
1.0
1.5
0
1
2
3
4
Follow-up Time (years)



Hazard ratio and 95% confidence interval for intensive systolic blood pressure treatment based on traditional Cox proportional hazard approach (red lines) and cumulative joint model approach (blue lines) for different subgroups: individuals with and without chronic kidney disease at baseline (A&B); women and men (C&D); black and non-black ethnicities (E&F); Individuals <75 and >=75 years of age (G&H); individuals with and without prevalent cardiovascular disease at baseline (I&J); baseline systolic blood pressure categories of 133-144 mmHg and <=132mmHg (K&L); subgroups with serious adverse events (M) and without serious adverse events (N) during follow-up. Orange vertical line denotes the time-point at which the statistical significance of the effect estimate is lost in each subgroup.

Supplemental Material

Statistical Analysis Plan and Supplemental Tables and Figures
(Framework Joint Models for Longitudinal and Time-to-Event Data)

This appendix has been provided by authors to give readers additional information about their work

Impact of Cumulative Systolic Blood Pressure and Serious Adverse Events on Efficacy of Intensive Blood Pressure Treatment: A randomized clinical trial.

SUPPLEMENTARY APPENDIX

Table of Contents

1. Definition of joint models for longitudinal and time-to-event data

To address research questions involving the association structure between repeated measures and event times, a class of statistical models has been developed known as joint models for longitudinal and time-to-event data[1]. Currently, the study of these models constitutes an active area of statistics research that has received a lot of attention in the recent years. In particular, after the early work on joint modeling approaches with application in AIDS research by Self and Pawitan[2] and DeGruttola and Tu [3], and the seminal papers by Faucett and Thomas [4] and Wulfsohn and Tsiatis [5] who introduced what could nowadays be called the standard joint model, there has been an explosion of developments in this field. Numerous papers have appeared proposing several extensions of the standard joint model including, among others, the cumulative joint model (cJM)[6,7], the flexible modeling of longitudinal trajectories, the incorporation of latent classes to account for population heterogeneity, the consideration of multiple longitudinal markers, modeling multiple failure times, and the calculation of dynamic predictions and accuracy measures.

2. Why applying joint models for longitudinal and to time-to-event data analysis to SPRINT trial?

SPRINT trial measured systolic blood pressure (SBP) monthly in the first trimester and then every quarter for 3.26 years median follow-up (range 0 to 4.5 years), in 9361 participants, achieving an average of 15 SBP measurements per person (range: 1-21) during follow-up[8]. This information was not taken into account in the primary SPRINT analysis. Given that: (1) this clinical trial evaluates a strategy of intensive pharmacological intervention (decreasing the SBP <120 mmhg) versus conventional treatment (SBP between 130-139 mmhg), (2) blood pressure is among the most important risk factor for major cardiovascular events (SPRINT primary outcome), (3) there is a high SBP variability within the subjects during follow-up (figure 1B), and (4) the occurrence of serious adverse events (SAEs) during follow-up can impact both the subsequent SBP figures as well as the primary outcome; a statistical analysis is required that evaluates the impact of longitudinal changes in SBP both within individuals and between intervention groups and takes also the cumulative effect of the SBP and the impact of the development of SAEs on the primary outcome into account. This analysis can only be achieved with a statistical model that includes all these elements at the same time, such as the cumulative joint model (cJM) analysis[9].

3. R studio version and packages for analysis

In the present analysis we used the statistical program R, under the console R studio version 3.2.5 (2016-04-14). The following packages of R software are required to be installed to perform the analysis: “shiny”, “nlme”, “lattice”, “lme4”, “MCMCglmm”, “geepack”, “MASS”, “corrplot”, “splines”, “Matrix”, “md5”, “survival”, “JM”

4. Formatting the database for the statistical analysis

The database must be converted to the long format, so that each row contains the different visits of each of the patients sequentially. The columns include the variables to study as shown in the following figure:

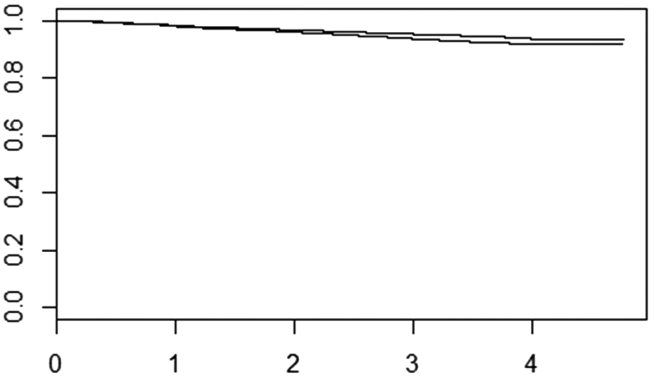
	id	indice	MASKID	timevisitday	INTENSIVE	sbp	EVENT_PRIM~Y	DAY_T_PRIM~Y	SAE_EVNT
129457	9360	1	S99992	0	1	154	0	737	0
129458	9360	2	S99992	30	1	118	0	737	0
129459	9360	3	S99992	60	1	108	0	737	0
129460	9360	4	S99992	90	1	118	0	737	0
129461	9360	5	S99992	180	1	134	0	737	0
129462	9360	6	S99992	270	1	118	0	737	0
129463	9360	7	S99992	360	1	112	0	737	0
129464	9360	8	S99992	450	1	115	0	737	0
129465	9360	9	S99992	540	1	119	0	737	0
129466	9360	10	S99992	630	1	115	0	737	0
129467	9360	11	S99992	720	1	109	0	737	0
129468	9361	1	S99997	0	1	146	0	1599	1
129469	9361	2	S99997	30	1	107	0	1599	1
129470	9361	3	S99997	60	1	97	0	1599	1
129471	9361	4	S99997	90	1	120	0	1599	1
129472	9361	5	S99997	180	1	109	0	1599	1
129473	9361	6	S99997	270	1	119	0	1599	1
129474	9361	7	S99997	360	1	106	0	1599	1
129475	9361	8	S99997	450	1	101	0	1599	1
129476	9361	9	S99997	540	1	93	0	1599	1
129477	9361	10	S99997	630	1	130	0	1599	1
129478	9361	11	S99997	720	1	124	0	1599	1
129479	9361	12	S99997	810	1	101	0	1599	1
129480	9361	13	S99997	900	1	117	0	1599	1
129481	9361	14	S99997	990	1	107	0	1599	1

5. Initial descriptive graphical analysis

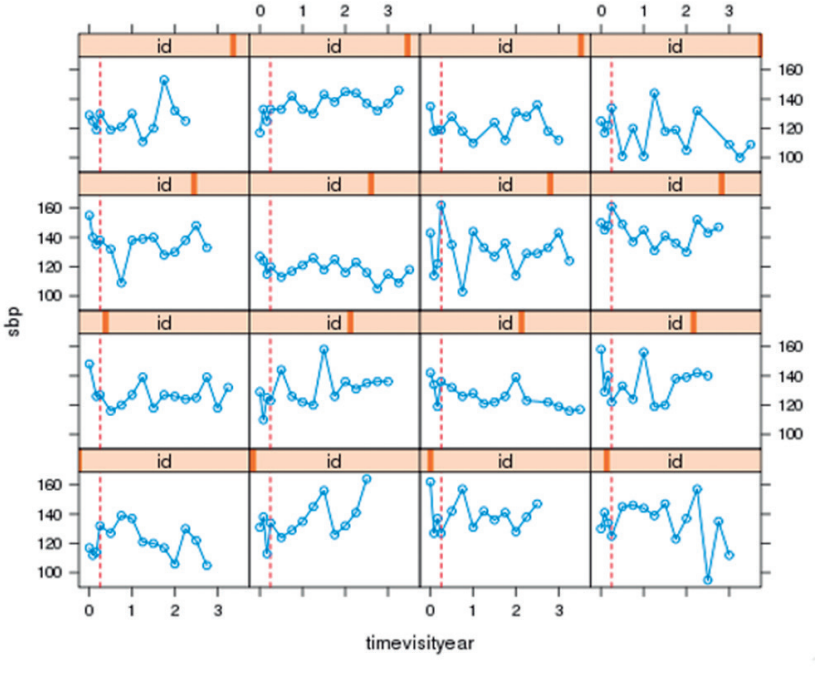
An initial Kaplan-Meier (KM) curve (figure 1A) and a plot of SBP variability within individuals in a random sample of participants were obtained. There is an important drop in SBP in the first 3 months of follow-up and a large SBP variability during the follow-up (figure 1B).

Figure 1. Kaplan Meier curve for the intensive and standard treatment groups (A) and plot of changes in systolic blood pressure in a random sample of SPRINT participants over time (intra-individual systolic blood pressure variability) (B)

A



B



Red dashed line represent the first three months of follow-up

6. Linear mixed model (LMM)

The LMM includes a fixed and a random part. The LMM fixed part takes into account the difference in the average SPB changes over time between the intensive and standard treatment groups. The LMM random part captures the SBP variability within individuals and can implicitly take into account unmeasured time-varying covariates (latent variables) that could affect this intra-individual variations, here this could be serious adverse events (SAEs).

We built a LMM that included SBP repeated measurements as dependent variable adjusted for treatment (intensive vs standard) and the interaction term between time and treatment. No other covariates were included in the model because both groups are balanced by randomization. To take into account the initial drop in SBP evolution, we set the knots appropriately (0.25, 0.5, 1.4 years). We also set the boundary knots (in this case the upper knot) not to the maximum (i.e., the default) but to the 95% percentile of the time variable (0, 3.5 year). We used a diagonal covariance matrix (pdDiag) in the random argument. The command to run LMM with the previously mentioned specifications using R software was:


```
fm2 <- lme(sbp ~ ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)) * INTENSIVE,
        data = LongData, method = "ML",
        random = list(id = pdDiag(form = ~ ns(timevisityear, k = c(0.25, 0.5, 1.4),
B = c(0, 3.5)))))
```

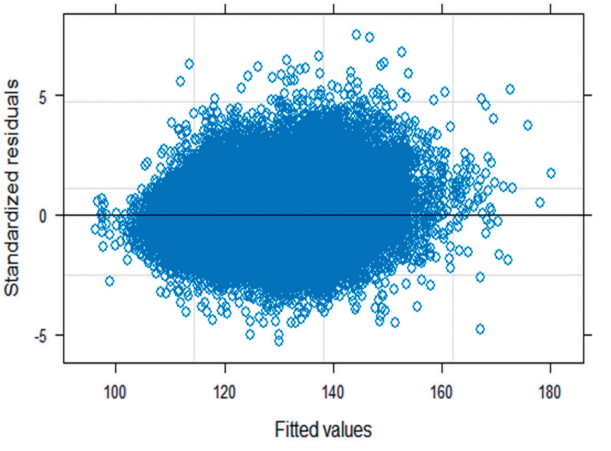
The result obtained in the analysis of the total population of SPRINT was:

```
> summary(fm2)
Linear mixed-effects model fit by maximum likelihood
 Data: LongData
      AIC      BIC    logLik
  1013459 1013615 -506713.6

Random effects:
 Formula: ~ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)) | id
 Structure: Diagonal
        (Intercept) ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1
StdDev:    6.121029                                                4.589185
        ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2
StdDev:                                                 6.835357
        ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3
StdDev:                                                 6.725934
        ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4 Residual
StdDev:                                                 6.168072 11.57985

Fixed effects: sbp ~ ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)) *      INTENSIVE

                                                                    Value Std.Error
(Intercept)                                                     137.98661 0.1747869
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1        -1.39380 0.2297875
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2        -0.48447 0.2284822
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3        -8.45465 0.4015053
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4         1.01689 0.2175400
INTENSIVE                                                       -0.59564 0.2466863
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1:INTENSIVE -13.23052 0.3241703
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2:INTENSIVE -10.62696 0.3212407
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3:INTENSIVE -25.34793 0.5662261
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4:INTENSIVE  -8.92699 0.3044276
```

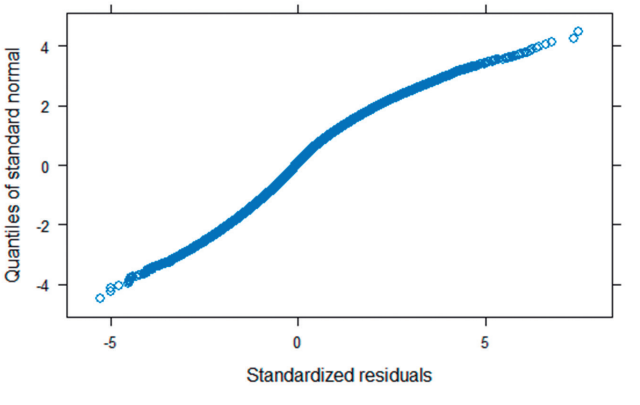
An evaluation of the residual distribution was obtained using the next commands: plot(fm2) and qqnorm(fm2)

Figure 2. Graphical analysis of residual distribution

Plot(fm2)



qqnorm(fm2)



7. Cox proportional hazard model

A traditional Cox proportional hazard model was built. This model only included treatment (intensive vs standard) as covariate. The commands used and results obtained were:

```
CoxFit2 <- coxph(Surv(YEAR_T_PRIMARY, EVENT_PRIMARY) ~ INTENSIVE,
            data = SurvData, x = TRUE)
```

```
> summary(CoxFit2)
Call:
coxph(formula = Surv(YEAR_T_PRIMARY, EVENT_PRIMARY) ~ INTENSIVE,
    data = SurvData, x = TRUE)

  n= 9068, number of events= 536

              coef exp(coef) se(coef)      z Pr(>|z|)
INTENSIVE -0.29364   0.74555  0.08722 -3.366 0.000761 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

          exp(coef) exp(-coef) lower .95 upper .95
INTENSIVE    0.7455      1.341    0.6284    0.8845

Concordance= 0.536  (se = 0.011 )
Rsquare= 0.001   (max possible= 0.651 )
Likelihood ratio test= 11.45  on 1 df,   p=0.0007161
Wald test            = 11.33  on 1 df,   p=0.0007613
Score (logrank) test = 11.41  on 1 df,   p=0.0007286
```

8. Standard joint model for longitudinal and time-to-event data

The LMM and Cox proportional hazards models were then joint by JM package of R software. The standard joint model assumes that the risk for an event at a particular time point *t* depends on the true level of the longitudinal marker at the same time point. The strength of the association between the current level of the marker and the risk is captured

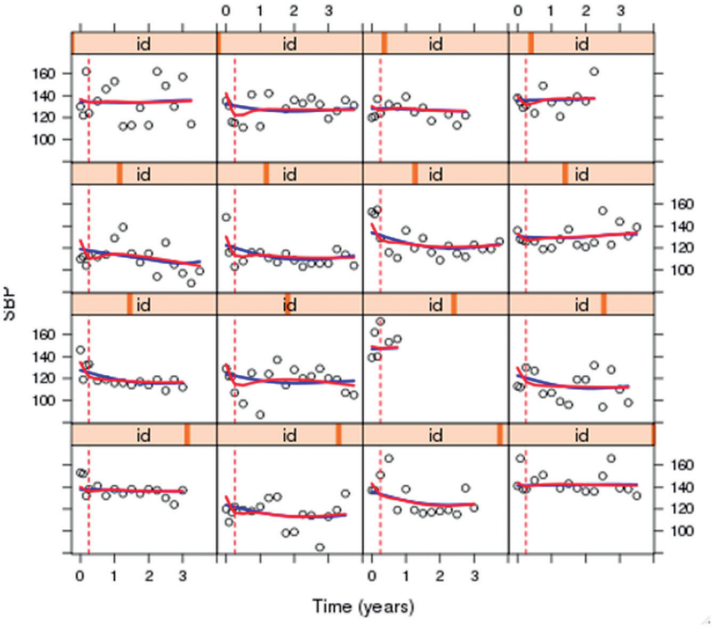
by the parameter α. In summary, the risk for an event at a specific time depends on features of the longitudinal trajectory at only a single time point. That is, from the entire history of the true marker levels $Mi(t) = \{mi(s),\ 0 \leq s < t\}$, the risk at time t is typically assumed to depend on either the marker level on the same time point $mi(t)$ or in a previous time point $mi(t - c)$.

We calculated and compared two models to find the best model fitted to the SPRINT data, taking into account the important drop in SBP during the first period of follow-up. Figure 3 show a comparison of fitted SBP data using these two models. The models under comparison were the next two LMMs:

```
fm1 <- lme(sbp ~ ns(timevisityear, 2) * INTENSIVE,
        data = LongData, method = "ML",
        random = list(id = pdDiag(form = ~ ns(timevisityear, knots = 2:3))))
fm2 <- lme(sbp ~ ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)) * INTENSIVE,
        data = LongData, method = "ML",
        random = list(id = pdDiag(form = ~ ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0,
3.5)))))
```

LMM fm1 used quadratic natural splines in the fixed part of the model and B-splines 2:3 in the random part of the model. The model fm2 was adjusted to take into account the drop in SBP during the first part of follow-up using more specific B-splines in both fixed and random parts. In conclusion, model fm2 fitted better into the changes in SBP over time than model fm1.

Figure 3. Plots for comparison between the two models fitted for systolic blood pressure



Blue line: fm1 model Red line: fm2

The output with the results of the standard joint model (jm2) are shown below:

```
Joint Model Summary:
Longitudinal Process: Linear mixed-effects model
Event Process: Relative risk model with piecewise-constant
  baseline risk function
Parameterization: Time-dependent

   log.Lik     AIC     BIC
 -509371.2 1018792 1018970

Variance Components:
                                                    StdDev
(Intercept)                                         6.1241
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1  4.5893
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2  6.8339
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3  6.7259
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4  6.1664
Residual                                           11.5799

Coefficients:
Longitudinal Process
                                                                  Value Std.Err  z-value p-value
(Intercept)                                                     137.9782  0.1748 789.3511 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1         -1.3786  0.2298  -5.9993 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2         -0.4606  0.2285  -2.0156  0.0438
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3         -8.4195  0.4016 -20.9657 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4          1.0531  0.2177   4.8365 <0.0001
INTENSIVE                                                        -0.5969  0.2467  -2.4195  0.0155
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1:INTENSIVE -13.2300  0.3241 -40.8167 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2:INTENSIVE -10.6369  0.3212 -33.1137 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3:INTENSIVE -25.3439  0.5662 -44.7624 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4:INTENSIVE  -8.9414  0.3045 -29.3680 <0.0001

Event Process
            Value Std.Err z-value p-value
INTENSIVE  0.1101  0.1330  0.8275  0.4080
Assoct     0.0283  0.0072  3.9263  0.0001
log(xi.1) -7.9297  1.0047 -7.8923
log(xi.2) -7.6117  0.9930 -7.6654
log(xi.3) -7.6211  0.9953 -7.6575
log(xi.4) -7.7285  0.9923 -7.7886
log(xi.5) -7.6262  0.9894 -7.7076
log(xi.6) -7.6893  0.9905 -7.7633
log(xi.7) -7.7292  0.9935 -7.7795

Integration:
method: (pseudo) adaptive Gauss-Hermite
quadrature points: 3

Optimization:
Convergence: 0

> exp(confint(jointFit2, parm = "Event"))
               2.5 %     est.   97.5 %
INTENSIVE 0.8601472 1.116361 1.448894
Assoct    1.0142957 1.028748 1.043407
```

9. Cumulative joint model (cJM) for longitudinal and time-to-event data

A common characteristic of all parameterizations we have seen so far is that they assume that the risk for an event at a specific time depends on features of the longitudinal trajectory at only a single time point. That is, from the entire history of the true marker levels $Mi(t) = \{mi(s),\ 0 \leq s < t\}$, the risk at time t is typically assumed to depend on either the marker level on the same time point $mi(t)$ or in a previous time point $mi(t - c)$, if lagged effects are considered. However, several authors have argued that this assumption is not always realistic, and in many cases we may benefit by allowing the risk to depend on a more elaborate function of the longitudinal marker history (Sylvestre and Abrahamowicz)[10], (Hauptmann et al)[11], (Vacek)[12].

One approach that allows the whole history of the marker to be associated with the hazard for an event is to include the integral of the longitudinal trajectory in the linear predictor of the relative risk submodel, representing the cumulative effect of the longitudinal outcome up to time point *t*. A graphical representation of this parameterization is given in figure 4. More specifically, the survival submodel takes the form:

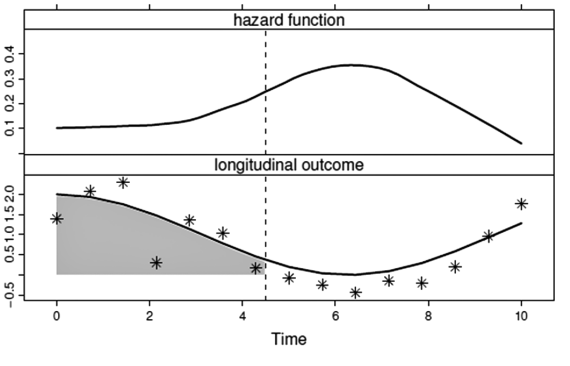
$$h_i(t) = h_0(t) \exp\left\{\gamma^\top w_i + \alpha \int_0^t m_i(s)\, ds\right\},$$

where for any particular time point *t*, α measures the strength of the association between the risk for an event at time point *t* and the area under the longitudinal trajectory up to the same time *t*, with the area under the longitudinal trajectory regarded as a suitable summary of the whole trajectory.

To fit a joint model under this parameterization, we can exploit the flexibility provided by the derivForm argument of jointModel() for the specification of an extra marker term to be added in the linear predictor of the survival submodel. In particular, instead of specifying the R formulas to define the time-dependent slope term *m'i*(*t*).

Therefore, to arrive at the cJM model in the SPRINT database, the integral of the longitudinal trajectory of SBP was included as the linear predictor of the relative risk sub-model. The integral represents the cumulative effect of the longitudinal trajectory of repeated SBP measurements up to time point t. The hazard ratio (HR) for the intensive treatment from the cJM approach, therefore, takes into account the cumulative impact of SBP changes over time.

Figure 4 Graphical concept of cumulative Joint Model for longitudinal and time-to-event data



The top panel illustrates the evolution of the hazard function in time, and the bottom panel shows that at each time point the entire area under the longitudinal trajectory is associated with the hazard.

The command used in R to obtain this cumulative joint model was:
jointFit2_cum <- update(jointFit2, parameterization = "slope", derivForm = iForm2)
summary(jointFit2_cum)
exp(confint(jointFit2_cum, parm = "Event"))

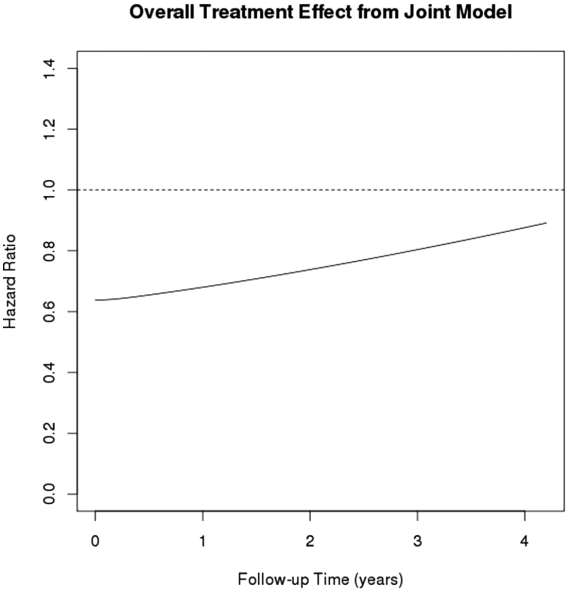
The output with the results of this cumulative joint model are shown below.

```
    derivForm = iForm2, knots = c(0.5, 1, 1.5, 2, 2.5, 3))

Data Descriptives:
Longitudinal Process  Event Process
Number of Observations: 128139 Number of Events: 536 (5.9%)
Number of Groups: 9068

Joint Model Summary:
Longitudinal Process: Linear mixed-effects model
Event Process: Relative risk model with piecewise-constant
  baseline risk function
Parameterization: Time-dependent slope

   log.Lik     AIC     BIC
 -509386.2 1018822 1019000

Variance Components:
                                                    StdDev
(Intercept)                                         6.1191
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1  4.5886
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2  6.8349
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3  6.7251
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4  6.1684
Residual                                           11.5801

Coefficients:
Longitudinal Process
                                                              Value Std.Err  z-value p-value
(Intercept)                                                137.9877  0.1748 789.5490 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1     -1.3947  0.2298  -6.0695 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2     -0.4880  0.2285  -2.1359  0.0327
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3     -8.4605  0.4015 -21.0724 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4      1.0097  0.2176   4.6401 <0.0001
INTENSIVE                                                    -0.5959  0.2467  -2.4160  0.0157
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))1:INTENSIVE -13.2294  0.3242 -40.8083 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))2:INTENSIVE -10.6248  0.3213 -33.0687 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))3:INTENSIVE -25.3455  0.5662 -44.7612 <0.0001
ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))4:INTENSIVE  -8.9248  0.3045 -29.3071 <0.0001

Event Process
           Value Std.Err  z-value p-value
INTENSIVE -0.4491  0.0945  -4.7549 <0.0001
Assoct.s  -0.0055  0.0016  -3.3792  0.0007
log(xi.1) -3.8185  0.1420 -26.8936
log(xi.2) -3.1521  0.2034 -15.4973
log(xi.3) -2.7900  0.2984  -9.3504
log(xi.4) -2.5370  0.3999  -6.3444
log(xi.5) -2.1029  0.4998  -4.2072
log(xi.6) -1.7698  0.6016  -2.9419
log(xi.7) -1.3678  0.7386  -1.8519

Integration:
method: (pseudo) adaptive Gauss-Hermite
quadrature points: 3

Optimization:
Convergence: 0

> exp(confint(jointFit2_cum, parm = "Event"))
               2.5 %      est.     97.5 %
INTENSIVE 0.5303476 0.6382004 0.7679864
Assoct.s  0.9913134 0.9944934 0.9976835
>
```

10. Plots of the hazard ratio changes over time for the primary PRINT outcome

We obtained the plots of the changes in the hazard ratios for the primary SPRINT primary outcome over time shown in figure 5. This graph shown us that hazard ratio for intensive treatment was losing its protective effect on the primary SPRINT outcome in the total population over time.

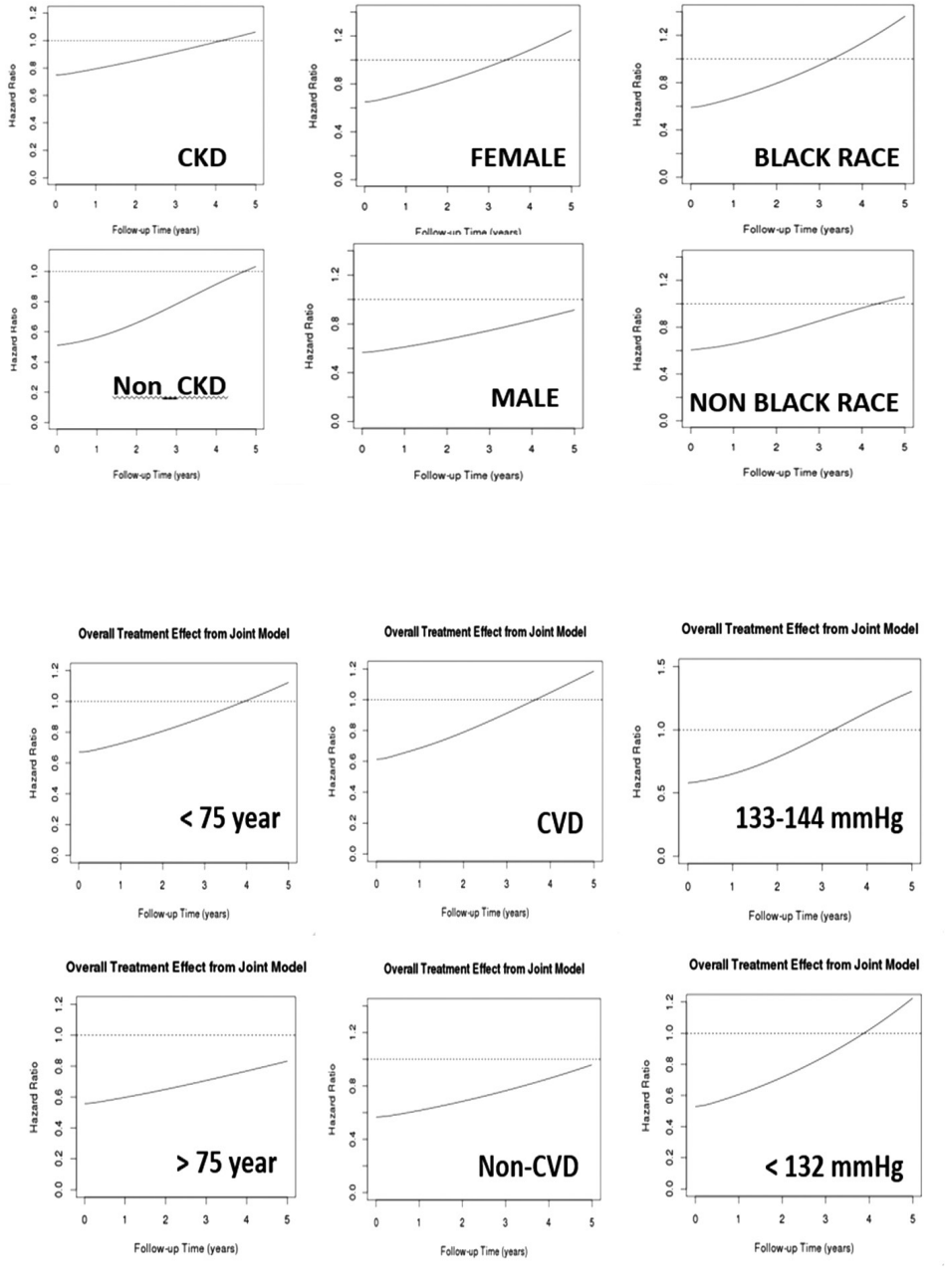
Figure 5 Changes in the hazard ratio for intensive treatment on the primary SPRINT outcome in the total population over time



11. Subgroup analysis based on the original SPRINT subgroups

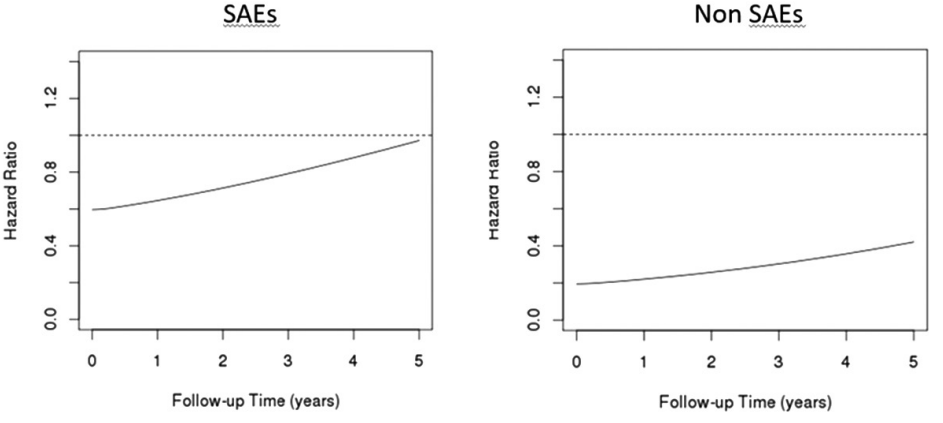
All of the previous stages were followed in the analysis of the subgroups. We considered the same subgroups as those investigated in the original SPRINT trial. We found the same tendency regarding losing the protective effect of intensive treatment on the primary SPRINT outcome in subgroup analysis. This was particularly the true for participants with previous chronic kidney disease or cardiovascular disease, women, individuals of Black ethnicity, those younger than 75 year, and participants with SBP >132 mmHg at baseline (Figure 6)

Figure 6. Plots of cumulative joint model analysis in the SPRINT subgroups



12. Subgroup analysis between SAEs vs non-SAEs groups

In the cJM, we analyzed the interaction between intensive treatment and SAEs in total population and in each of the subgroups under study and we found statistically significance association (P< 0.0001). Therefore, we stratified the cJM analyses based on occurrence of SAEs during follow-up. We found that the protective effect of intensive treatment in participants who suffered SAEs was lower (higher HR) than non-SAEs participants. At the same time, the protective effect was lost earlier in SAEs participants than non-SAEs participants (Figure 7)

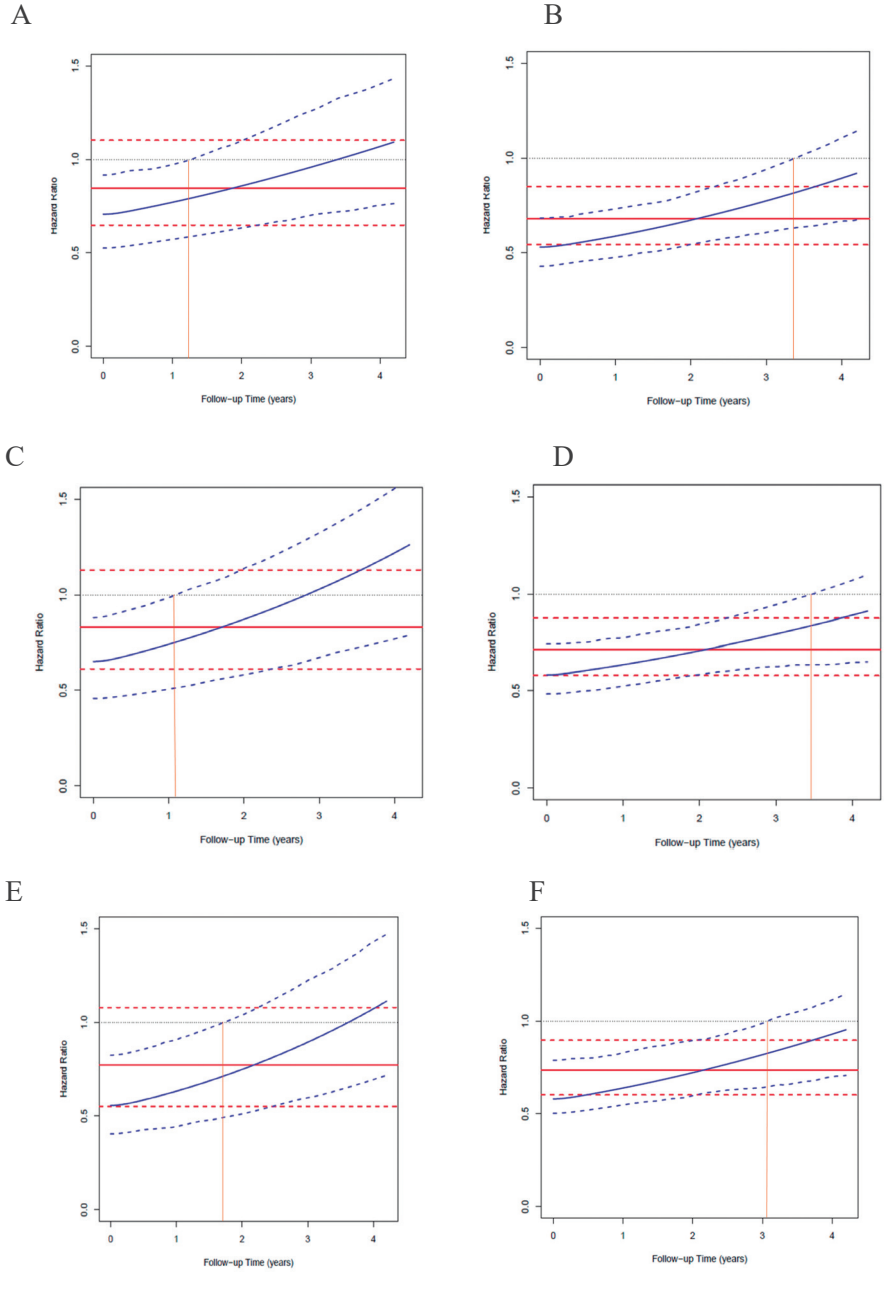


13. Bootstrapping to create summary plots for simultaneous comparison between Cox proportional hazard model and cumulative joint model

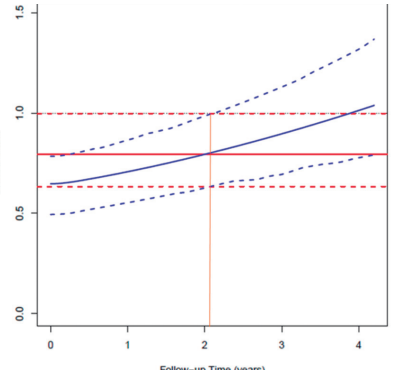
Finally, we ran bootstrapping with 400 repetitions in order to obtain the 95% confidence intervals for all plots obtained in our analysis. We included both Cox proportional hazard plots and cumulative joint models in the same graph. In order to facilitate the interpretation of our results, we added an orange vertical line to the graph indicating the time when the confidence interval of cumulative joint model crossed the level of non-statistical significance (HR=1.0)

All finals plots comparing the changes in the hazard ratio over time using the traditional Cox proportional model versus the cumulative hazard model are shown below. These graphs represent the hazard ratio and 95% confidence interval for intensive systolic blood pressure treatment based on traditional Cox proportional hazard approach (red lines) and cumulative joint model approach (blue lines) for different subgroups: individuals with and without chronic kidney disease at baseline (A&B); women

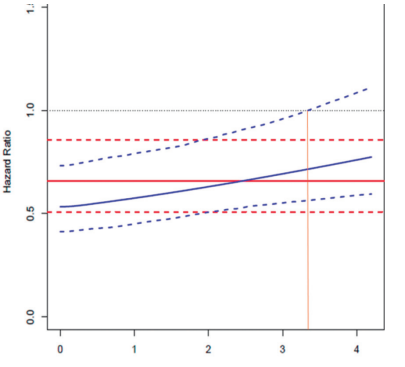
and men (C&D); black and non-black ethnicities (E&F); Individuals <75 and >=75 years of age (G&H); individuals with and without prevalent cardiovascular disease at baseline (I&J); baseline systolic blood pressure categories of 133-144 mmHg and <=132mmHg (K&L). Orange vertical line denotes the time point at which the statistical significance of the effect estimate is lost in each subgroup.



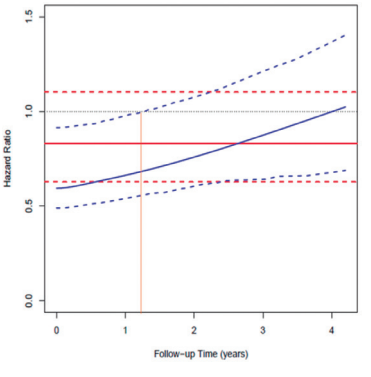
G


1.5
1.0
0.5
0.0
0
1
2
3
4

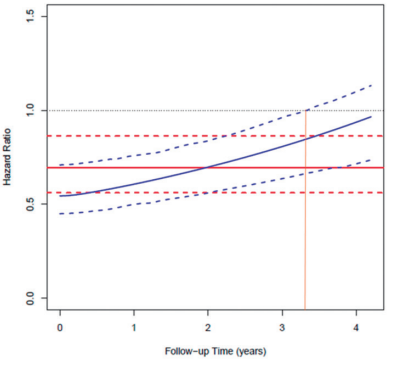
H


Hazard Ratio
1.0
0.5
0.0
0
1
2
3
4

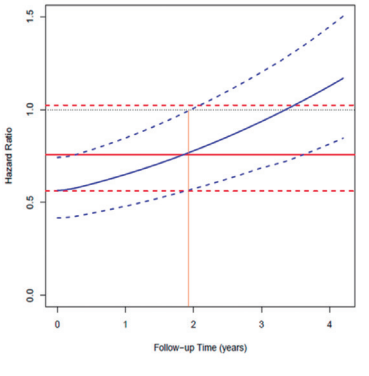
I


Hazard Ratio
1.5
1.0
0.5
0.0
0
1
2
3
4
Follow-up Time (years)

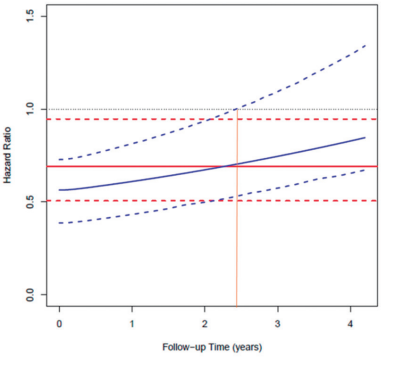
J


Hazard Ratio
1.5
1.0
0.5
0.0
0
1
2
3
4
Follow-up Time (years)

K


Hazard Ratio
1.5
1.0
0.5
0.0
0
1
2
3
4
Follow-up Time (years)

L


Hazard Ratio
1.5
1.0
0.5
0.0
0
1
2
3
4
Follow-up Time (years)

14. R software script

```
###STAGES JOINT MODEL ANALYSIS SBP

1. ### Install packages needed

## Longitudinal data Analysis
install.packages(c("shiny", "nlme", "lattice", "lme4",
                   "MCMCglmm", "geepack", "MASS", "corrplot", "splines", "Matrix", "md5"),
                 dependencies = TRUE)

library("Rcpp")
library("Matrix")
library("lme4")
library("lattice")
library("lme4")
library("MASS")
library("splines")
library("geepack")
library("Matrix")
library("corrplot")
library("MCMCglmm")
library("shiny")
library("nlme")
library("coda")
library("ape")

library(FinTS)
package.dir('nlme')

## Survival Data Analysis
install.packages("survival")
library("survival")

### Starting second analysis professor Rizopoulos
install.packages("JM")

library("JM")
library("lattice")
load("Longall_17.RData")
```

```
LongData <- Longall_17[c("id", "sbp", "timevisityear", "INTENSIVE", "YEAR_T_
PRIMARY", "EVENT_PRIMARY")]
LongData <- LongData[LongData$timevisityear < LongData$YEAR_T_PRIMARY, ]
LongData <- LongData[complete.cases(LongData), ]
LongData <- with(LongData, LongData[order(id, timevisityear), ])
SurvData <- LongData[!duplicated(LongData$id), ]

#########################################################################
############################################################
```

2. # Descriptive analysis

```
plot(survfit(Surv(YEAR_T_PRIMARY, EVENT_PRIMARY) ~ INTENSIVE, data =
SurvData))

ids <- sample(LongData$id, 16)
xyplot(sbp ~ timevisityear | id, data = LongData, subset = id %in% ids,
      type = "b", abline = list(v = 0.25, lty = 2, col = 2))


#########################################################################
############################################################
```

3. ## Linear mixed model

```
# account for initial drop in SBP evolution by setting the knots appropriatelly;
# it is also a good idea to set the boundary knots (in this case the upper knot) not to
# the maximum (i.e., the default) but to the 95% percentile of the time variable

fm2 <- lme(sbp ~ ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)) * INTENSIVE,
          data = LongData, method = "ML",
          random = list(id = pdDiag(form = ~ ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0,
3.5)))))
summary(fm2)

plot(fm2)
qqnorm(fm2)
#########################################################################
############################################################
```

4.## Cox model

```
CoxFit2 <- coxph(Surv(YEAR_T_PRIMARY, EVENT_PRIMARY) ~ INTENSIVE,
          data = SurvData, x = TRUE)

summary(CoxFit2)

##########################################################################
############################################################
```

5.## Standard Joint Model using fm2

```
jointFit2 <- jointModel(fm2, CoxFit2, timeVar = "timevisityear",
                method = "piecewise-PH-aGH", knots = c(0.5, 1, 1.5, 2, 2.5, 3))

summary(jointFit2)
exp(confint(jointFit2, parm = "Event"))

# Overall treatment effect (JM STANDARD POINT)

JMFit <- jointFit2 # select the joint model (only works for jointFit1 & jointFit2)

ND1 <- expand.grid(timevisityear = seq(0, 4.2, length.out = 100),
          INTENSIVE = 1, sbp = 100, YEAR_T_PRIMARY = 4.2, EVENT_PRIMARY = 1)
ND0 <- expand.grid(timevisityear = seq(0, 4.2, length.out = 100),
          INTENSIVE = 0, sbp = 100, YEAR_T_PRIMARY = 4.2, EVENT_PRIMARY = 1)

fixed1 <- c(model.matrix(JMFit$termsYx, ND1) %*% fixef(JMFit))
fixed0 <- c(model.matrix(JMFit$termsYx, ND0) %*% fixef(JMFit))
base1 <- c(model.matrix(JMFit$termsT, ND1)[, -1, drop= FALSE] %*% fixef(JMFit,
"Event")[c("INTENSIVE")])
base0 <- c(model.matrix(JMFit$termsT, ND0)[, -1, drop= FALSE] %*% fixef(JMFit,
"Event")[c("INTENSIVE")])
alpha <- fixef(JMFit, "Event")["Assoct"]
logHR <- base1 + alpha * fixed1 - base0 - alpha * fixed0
plot(ND1$timevisityear, exp(logHR), type = "l",
    ylab = "Hazard Ratio", xlab = "Follow-up Time (years)",
    main = "Overall Treatment Effect from Joint Model")
abline(h = 1, lty = 2)
```

```
##########################################################################
##########################################################
```

6. Plots of comparison between LMM model fm1 and model fm2

```
# check fit of the models in the individual trajectories

LongData$fitted1 <- fitted(fm1, level = 1)
LongData$fitted2 <- fitted(fm2, level = 1)

ids <- sample(LongData$id, 16) # a random sample of 16 patients
xyplot(sbp + fitted1 + fitted2 ~ timevisityear | id, data = LongData,
      panel = function (x, y, ...) {
         x.mat <- matrix(x, ncol = 3)
         y.mat <- matrix(y, ncol = 3)
         panel.xyplot(x.mat[, 1], y.mat[, 1], type = "p", col = "black")
         panel.xyplot(x.mat[, 2], y.mat[, 2], type = "l", lwd = 2, col = "blue")
         panel.xyplot(x.mat[, 3], y.mat[, 3], type = "l", lwd = 2, col = "red")
         panel.abline(v = 0.25, lty = 2, col = 2)
      }, subset = id %in% ids, layout = c(4, 4), as.table = TRUE,
      xlab = "Time (years)", ylab = "SBP")


##########################################################################
##########################################################
```

7. Cummulative joint model using fm2

```
## cumulative effect (area) Joint Model using fm2

iForm2 <- list(fixed = ~ 0 + timevisityear
            + ins(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))
            + timevisityear:INTENSIVE
            + ins(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)):INTENSIVE,
            indFixed = seq_along(fixef(fm2)),
            random = ~ 0 + timevisityear
            + ins(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)),
            indRandom = seq_len(ncol(ranef(fm2))))

jointFit2_cum <- update(jointFit2, parameterization = "slope", derivForm = iForm2)
```

```
summary(jointFit2_cum)
exp(confint(jointFit2_cum, parm = "Event"))

#########################################################################
############################################################
```

8. Plots of the hazard ratio changes over time for the SPRINT primary outcome

```
# Overall treatment effect (JM cumulate)

JMFit <- jointFit2_cum # select the joint model (only works for jointFit1 & jointFit2)

ND1 <- expand.grid(timevisityear = seq(0, 4.2, length.out = 100),
          INTENSIVE = 1, sbp = 100, YEAR_T_PRIMARY = 4.2, EVENT_PRIMARY = 1)
ND0 <- expand.grid(timevisityear = seq(0, 4.2, length.out = 100),
          INTENSIVE = 0, sbp = 100, YEAR_T_PRIMARY = 4.2, EVENT_PRIMARY = 1)

fixed1 <- c(model.matrix(JMFit$termsYx.deriv, ND1) %*% fixef(JMFit)
[JMFit$derivForm$indFixed])
fixed0 <- c(model.matrix(JMFit$termsYx.deriv, ND0) %*% fixef(JMFit)
[JMFit$derivForm$indFixed])
base1 <- c(model.matrix(JMFit$termsT, ND1)[, -1, drop= FALSE] %*% fixef(JMFit,
"Event")[c("INTENSIVE")])
base0 <- c(model.matrix(JMFit$termsT, ND0)[, -1, drop= FALSE] %*% fixef(JMFit,
"Event")[c("INTENSIVE")])
alpha <- fixef(JMFit, "Event")["Assoct.s"]
logHR <- base1 + alpha * fixed1 - base0 - alpha * fixed0
plot(ND1$timevisityear, exp(logHR), type = "l",
    ylab = "Hazard Ratio", xlab = "Follow-up Time (years)",
    main = "Overall Treatment Effect from Joint Model", ylim = c(0, 1.4))
abline(h = 1, lty = 2)

#####################################################################
#############################################################
```

9. Comparison plots between traditional Cox proportional hazard model and cumulative joint model

 # Bootstrap CI for overall treatment effect

bootstrap_cumJM

```
# Functions
run_boot <- function (m) {
 library("JM")
   load("C:/Users/dimitris/Documents/Students/Oscar/Longall_17.RData")  #  CHANGE
THIS to get data from location on server
  LongData <- Longall_17[c("id", "sbp", "timevisityear", "INTENSIVE", "FEMALE",
"AGE",
                         "YEAR_T_PRIMARY", "EVENT_PRIMARY")]
 LongData <- LongData[LongData$timevisityear < LongData$YEAR_T_PRIMARY, ]
 LongData <- LongData[complete.cases(LongData), ]
 LongData <- with(LongData, LongData[order(id, timevisityear), ])
 # function to run model
 fit_cumJM <- function (LongData, SurvData) {
  # Fit cumulative joint model
  lmeFit <- lme(sbp ~ ns(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)) * INTENSIVE,
                data = LongData, method = "ML",
               random = list(id_new = pdDiag(form = ~ ns(timevisityear, k = c(0.25, 0.5, 1.4),
                                                 B = c(0, 3.5)))))
   CoxFit <- coxph(Surv(YEAR_T_PRIMARY, EVENT_PRIMARY) ~ INTENSIVE,
                   data = SurvData, x = TRUE)
   iForm <- list(fixed = ~ 0 + timevisityear
                 + ins(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5))
                 + timevisityear:INTENSIVE
                 + ins(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)):INTENSIVE,
                 indFixed = seq_along(fixef(lmeFit)),
                 random = ~ 0 + timevisityear
                 + ins(timevisityear, k = c(0.25, 0.5, 1.4), B = c(0, 3.5)),
                 indRandom = seq_len(ncol(ranef(lmeFit))))
   JMFit <- jointModel(lmeFit, CoxFit, timeVar = "timevisityear",
                       method = "piecewise-PH-aGH", knots = c(0.5, 1, 1.5, 2, 2.5, 3),
                       parameterization = "slope", derivForm = iForm)
   # Overall treatment effect for cumulative effect (area)
   ND1 <- expand.grid(timevisityear = seq(0, 4.2, length.out = 100),
           INTENSIVE = 1, sbp = 100, YEAR_T_PRIMARY = 4.2, EVENT_PRIMARY = 1)
   ND0 <- expand.grid(timevisityear = seq(0, 4.2, length.out = 100),
           INTENSIVE = 0, sbp = 100, YEAR_T_PRIMARY = 4.2, EVENT_PRIMARY = 1)
```

```
        fixed1 <- c(model.matrix(JMFit$termsYx.deriv, ND1) %*% fixef(JMFit)
[JMFit$derivForm$indFixed])
        fixed0 <- c(model.matrix(JMFit$termsYx.deriv, ND0) %*% fixef(JMFit)
[JMFit$derivForm$indFixed])
    base1 <- c(model.matrix(JMFit$termsT, ND1)[, -1, drop= FALSE] %*% fixef(JMFit,
"Event")[c("INTENSIVE")])
    base0 <- c(model.matrix(JMFit$termsT, ND0)[, -1, drop= FALSE] %*% fixef(JMFit,
"Event")[c("INTENSIVE")])
   alpha <- fixef(JMFit, "Event")["Assoct.s"]
   logHR <- base1 + alpha * fixed1 - base0 - alpha * fixed0
   exp(logHR)
  }
  # create Bootstrap sample
  create_boot_data <- function (data, idVar, seed = 1L) {
   set.seed(seed)
   ids <- unique(data[[idVar]])
   samp_ids <- sample(ids, replace = TRUE)
   out_data <- vector("list", length(ids))
   for (i in seq_along(ids)) {
    DD <- data[data[[idVar]] == samp_ids[i], ]
    DD$id_new <- i
    out_data[[i]] <- DD
   }
   rm(list = ".Random.seed", envir = globalenv())
   do.call("rbind", out_data)
  }
  LongData_boot <- create_boot_data(data = LongData, idVar = "id", seed = m)
  SurvData_boot <- LongData_boot[!duplicated(LongData_boot$id_new), ]
  # fit joint model and extract results
  fit_cumJM(LongData_boot, SurvData_boot)
}

##########################################################################
############################################################
```

Supplementary Tables and Figures

Table S1. Hazard Ratio Based on Cumulative Joint Model Approach at the Start and End of Follow-Up in the Total SPRINT Population and among Different Subgroups

	cJM (HR CI 95%)	
	Start Point	**End point**
Total population	0.60 (0.50, 0.72)*	0.89 (0.69, 1.10)
Sub-groups		
Baseline CKD		
CKD	0.75 (0.56, 0.99)*	1.09 (0.76, 1.43)
Non- CKD	0.53 (0.42, 0.68)*	0.91 (0.67, 1.14)
Gender		
Female	0.65 (0.47, 0.91)*	1.26 (0.78, 1.61)
Male	0.57 (0.45, 0.71)*	0.91 (0.65, 1.1)
Ethnicity		
Black	0.59 (0.40, 0.85)*	1.11 (0.72, 1.47)
Non- black	0.61 (0.49, 0.75)*	0.95 (0.71, 1.15)
Age		
< 75	0.67 (0.52, 0.86)*	0.89 (0.79, 1.37)
>= 75	0.56 (0.42, 0.74)*	0.77 (0.59, 1.11)
Baseline CVD		
CVD	0.68 (0.50, 0.92)*	1.02 (0.69, 1.4)
Non-CVD	0.57 (0.45, 0.72)*	0.97 (0.74, 1.13)
Baseline SBP		
<=132	0.53 (0.38, 0.75)*	0.85 (0.67, 1.34)
133-144	0.57 (0.41, 0.79)*	1.17 (0.85, 1.50)
>= 145	0.70 (0.52, 0.94) *	0.84 (0.70, 1.41)
Serious adverse events		
SAEs	0.60 (0.50, 0.72)*	0.94 (0.67, 1.34)
Non-SAEs	0.19 (0.06, 0.63)*	0.40 (0, 1.02)

CI denotes confidence interval, cJM cumulative joint model, CKD chronic kidney disease, CVD cardiovascular disease, HR hazard ratio, SAEs serious adverse events, and SBP systolic blood pressure.

*P < 0.05

Table S2. Distribution of Specific Serious Adverse Events for the Intensive and Standard Treatment Categories in the Total SPRINT Population and among Different Sub-Groups

	HIPOTA ER/ SAE	AKI ER/ SAE	ELEC- TROLYTES	FALLS ER/ SAE	SYNCOPE ER/SAE	BRADICARDIA	N participants
Total population							
Intensive	157 (3.45)*	203 (4.46)*	175 (3.85)*	329 (7.23)	159 (3.49)*	102 (2.24)	4552
Standard	91 (2.02)	117 (2.59)	123 (2.72)	323 (7.15)	112 (2.48)	80 (1.77)	4516
Sub-groups							
Baseline CKD							
CKD intensive	59 (4.55)	116 (8.95)*	71 (5.48)	132 (10.19)	58 (4.48)	39 (3.01)	1296
CKD standard	44 (3.49)	77 (6.10)	50 (3.96)	133 (10.54)	44 (3.49)	41 (3.25)	1262
Non- CKD intensive	98 (3.01)*	87 (2.67)*	104 (3.2)†	197 (6.05)	101 (3.10)†	63 (1.94)†	3254
Non- CKD standard	47 (1.44)	40 (1.23)	73 (2.24)	150 (5.84)	68 (2.09)	39 (1.20)	3253
Gender							
Female intensive	43 (2.65)	53 (3.26)†	97 (5.97)*	167 (10.28)	62 (3.82)†	29 (1.78)	1625
Female standard	28 (1.77)	30 (1.9)	57 (3.6)	136 (8.6)	38 (2.4)	27 (1.71)	1582
Male intensive	114 (3.9)*	150 (5.13)*	78 (2.67)	162 (5.54)	97 (3.32)	73 (2.5)	2925
Male standard	63 (2.15)	87 (2.97)	66 (2.25)	187 (6.38)	74 (2.52)	53 (1.81)	2933
Ethnicity							
Black intensive	44 (3.12)†	80 (5.67)*	51 (3.61)	73 (5.17)	46 (3.26)	24 (1.7)	1412
Black standard	26 (1.81)	42 (2.92)	45 (3.13)	61 (4.24)	32 (2.23)	16 (1.11)	1438
Non- black intensive	113 (3.6)*	123 (3.92)*	124 (3.95)*	256 (8.16)	113 (3.6)†	78 (2.49)	3138
Non-black standard	65 (2.11)	75 (2.44)	78 (2.53)	262 (8.51)	80 (2.6)	64 (2.08)	3077

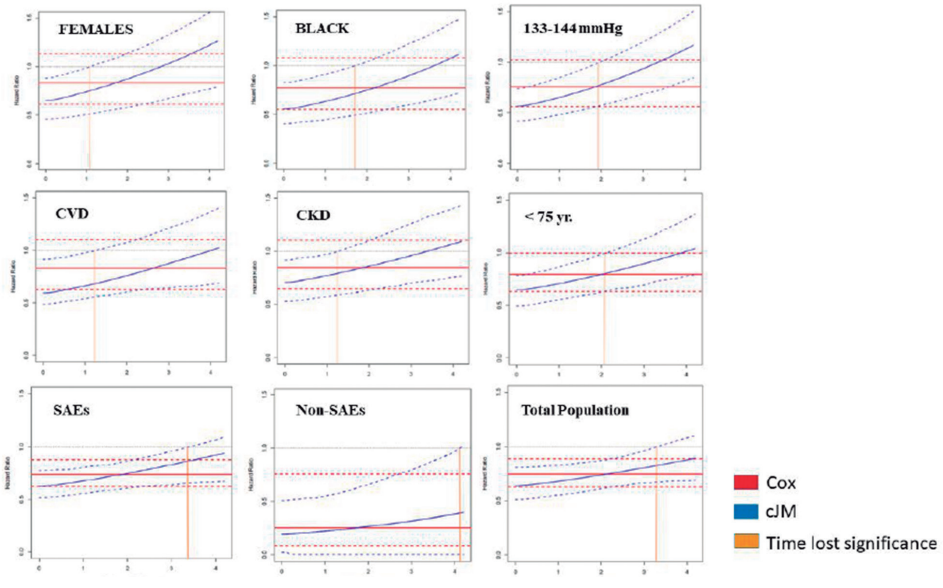
	HIPOTA ER/ SAE	AKI ER/ SAE	ELEC- TROLYTES	FALLS ER/ SAE	SYNCOPE ER/SAE	BRADICARDIA	N participants
Age							
< 75 intensive	108 (3.3)*	126 (3.85)*	110 (3.36)†	174 (5.31)†	97 (2.96)*	54 (1.65)†	3275
< 75 standard	56 (1.72)	64 (1.96)	77 (2.36)	136 (4.17)	62 (1.9)	31 (0.95)	3258
>= 75 intensive	49 (3.84)	77 (6.04)†	65 (5.1)	155 (12.16)	62 (4.86)*	48 (3.76)	1275
>= 75 standard	35 (2.78)	53 (4.22)	46 (3.66)	187 (14.88)†	50 (3.98)	49 (3.9)	1257
Baseline CVD							
CVD intensive	44 (4.78)†	60 (6.51)†	47 (5.1)†	76 (8.25)	37 (4.02)	37 (4.02)	921
CVD standard	27 (2.98)	38 (4.2)	26 (2.87)	76 (8.4)	31 (3.43)	26 (2.87)	905
Non-CVD intensive	113 (3.11)*	143 (3.94)*	128 (3.53)†	253 (6.97)	122 (3.36)*	65 (1.79)	3629
Non-CVD standard	64 (1.77)	79 (2.19)	97 (2.69)	247 (6.84)	81 (2.24)	54 (1.5)	3610
Baseline SBP							
<=132 intensive	55 (3.57)*	56 (3.63)	31 (2.08)	107 (6.94)	56 (3.63)†	32 (2.08)	1542
<= 132 standard	29 (1.95)	36 (2.42)	57 (3.7)*	97 (6.51)	31 (2.08)	25 (1.68)	1490
133-144 intensive	47 (3.24)*	68 (4.69)*	49 (3.38)†	110 (7.58)	43 (2.96)	34 (2.34)	1451
133-144 standard	26 (1.73)	32 (2.13)	30 (1.99)	115 (7.65)	35 (2.33)	21 (1.4)	1504
>= 145 intensive	55 (3.53)	79 (5.07)†	62 (4.08)	112 (7.19)	60 (3.85)	36 (2.31)	1557
>= 145 standard	36 (2.37)	49 (3.22)	49 (3.38)	111 (7.3)	46 (3.02)	34 (2.24)	1521

CKD: Chronic Kidney Disease; Non-CKD: Non Chronic Kidney Disease; CVD: Cardiovascular Disease; Non-CVD: Non Cardiovascular Disease; SAEs: Serious Adverse Events; Non_SAES: Non Serious Adverse Events.

*P value for the difference between intensive and standard treatment < 0.01

† P value for the difference between intensive and standard treatment < 0.05

Figure S1. Summary of the main results: Changes in the hazard ratio over time in the SPRINT subgroup that lost early benefits of intensive treatment.



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CHAPTER 2.3.1

Reply to editorial letter by Paul K. Whelton and David Reboussin. Joint modeling of systolic blood pressure and the primary outcome in SPRINT

Published in: Journal of Hypertension 37(8): 1729-1730, August 2019. in response to: Reboussin DM, Whelton PK. Joint modeling of systolic blood pressure and the primary outcome in systolic blood pressure intervention trial. Journal of Hypertension 2019, 37: 1729-1733.

Dear Editor:

We would to thank Dr. Reboussin and Dr. Whelton, SPRINT main investigators for reading our secondary analysis of the SPRINT trial[1,2], and their comments in our analysis in the letter to the Editor.

We agree with Dr. Reboussin and Dr. Whelton that conditioning on post randomization variables can lead to challenges in the interpretation of the treatment effect. This why we have selected to work with the joint modeling approach that does *not* condition on systolic blood pressure (SBP) after randomization but rather treats it as an outcome. In particular, the joint model uses a linear mixed model for SPB that explicitly allows for different SPB profiles in the two treatment groups. In addition, among others it accounts for the correlations in the repeated SBP measurements per patient, for missing at random missing data, and the endogenous nature of SPB that relate to the challenges in the interpretation mentioned by Dr. Reboussin and Dr. Whelton.

We also agree with Dr. Reboussin and Dr. Whelton that the total effect of an intervention is the sum of its effects on the outcome through all mechanisms and can be thought of as having two components: an indirect effect, in SPRINT the portion produced through changes in SBP, and a direct effect, in SPRINT the portion not produced by changes in SBP. This is why our proportional hazards model for the primary outcome contains two separate coefficients for the direct and indirect effects. More specifically, our model is defined mathematically as follows:

$$SBP_i(time) = f(time, \beta, Treat) + measurement\_error_i$$
$$h_i(time) = h_0(time) \exp[\Upsilon \cdot Treat + \alpha \cdot cumulative\{f(time, \beta, Treat)\}]$$

The first model is the linear mixed model for SBP, which, as mentioned above, explicitly accounts for different SBP evolutions per treatment group via function f(time, β, Treat). The coefficients β quantify the difference between the treatment groups in SBP over time.

The second model is the proportional hazards model for the primary endpoint; h_i(time) denote the hazard function of patient i, and h_0(time) is the baseline hazard. The indirect treatment effect produced through changes in SBP over time is capture in term "α · cumulative{f(time, β, Treat)}", where α is the coefficient quantifying the strength of the indirect association. The direct (residual) treatment effect is the term "ϒ · Treat", where ϒ is the coefficient quantifying the strength of the direct association. The total effect we have reported is the sum of the two[3,] (Figure 1).

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Disclosures: None

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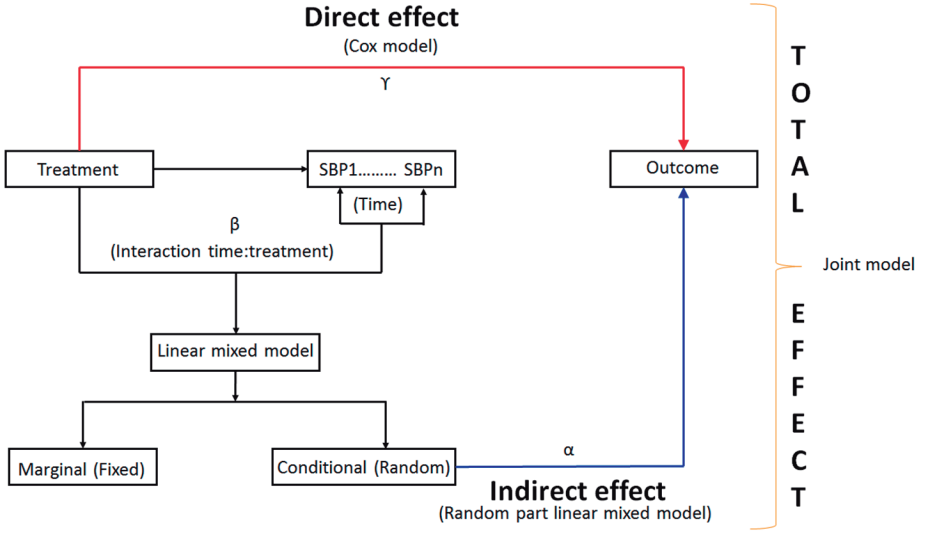

Figure 2. SBP1: First measurement of systolic blood pressure; SBPn: last measurement of systolic blood pressure. (Complete history of all SBP measurements previous to primary SPRINT outcome and its area under the curve (cumulative SBP) are being taken into account in the cumulative joint model analysis)

CHAPTER 2.3.2

Editorial letter to Circulation: Letter by Rueda-Ochoa et al regarding article, "Potential Cardiovascular Disease Events Prevented With Adoption of the 2017 American College of Cardiology/ American Heart Association Blood Pressure Guideline"

Published in: Circulation 2019;139:e1019-e1020. DOI: 10.1161/ CIRCULATIONAHA. 118.039332

To the Editor:

The recent article by Bress AP, et al[1], regarding the potential cardiovascular events prevented with adoption of the 2017 American College of Cardiology (ACC)/ American Heart Association (AHA) blood pressure guidelines, reports that the implementation of the new guidelines for management of hypertension[2] could prevent 3 million new major adverse cardiovascular events (MACE) per 10-years, but at the same time increase the serious adverse events (SAEs) associated with this intervention by 3.3 million per 10-years. The authors conclude that the benefits are greater than the risks of implementing intensive blood pressure treatment based on the scientific evidence published to date.

The new 2017 ACC/AHA guidelines for management of hypertension is predominantly based on the results of the SPRINT study (Systolic Blood Pressure Intervention Trial). It is worth mentioning that a previous analyses by Dr. Bress and colleagues regarding implementation of the SPRINT in the National Health and Nutrition Examination Survey (NHANES) 1999-2006 population, showed a reduction of 107,500 deaths per-year with intensive blood pressure lowering. However, an increase in the SAEs (N: 222,600 per-year), produced by this intervention, was more than twice the benefits[3].

In addition, the authors of the present study[1] argue that SAEs are predominantly mild, not fatal, and have a complete recovery within the first 12 months. We recently published a secondary analysis of the SPRINT study[4]. We found that although the number of SAEs was similar in the intensive treatment compared to the standard treatment group, the SAEs produced in the intensive treatment arm were more severe and increased the risk of the primary SPRINT outcome three times more compared with the SAEs produced in the standard treatment group. Our analyses further indicated an impact of SAEs on the sustainability of treatment benefit over time, leading to earlier loss of beneficial effect of treatment during follow-up among patients suffering SAEs compared to those who did not suffer SAEs. We also found that intensive treatment lost its beneficial impact earlier in several subgroups including women, individuals of black ancestry, and patients with prevalent cardiovascular disease or chronic kidney disease at baseline. Similarly,

another study recently published by Chi G et al[5] showed that the intensive treatment strategy does not reach a net clinical benefit when weighing the benefit of MACE reduction against the risk of increase in SAEs.

In our view, the ongoing discussion regarding the net clinical benefit of intensive blood pressure treatment highlights the need for a clinical trial with a longer period of follow-up than that achieved in the SPRINT study. Such a trial would need to also address the sustainability of intensive treatment over time, the safety of patients, and the effectiveness of treatment among different subgroups. This all is in order to comply with one of the most important premises of the medicine tenet "Primum non nocere".

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Disclosures: None

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CHAPTER 3

Advanced methods for causal inference

CHAPTER 3A

Propensity score matching to use population-based studies as controls for interventional studies

CHAPTER 3A.1

Survival after uncomplicated EVAR in octogenarians is similar to the general population of octogenarians without an abdominal aortic aneurysm

Poster presentation in: Caritas international Congress 2018 - London - UK

Poster presentation in: The European Society for Vascular Surgery 33rd Annual Meeting 2019. Published in: European Journal of Vascular and Endovascular Surgery. Volume 58, Issue 6 Supplement 3 e605-e606 December 2019. https://doi.org/10.1016/j.ejvs.2019.09.086

Published in Eur J Vasc Endovasc Surg 2020;59(5): 740-747

WHAT THIS PAPER ADDS

Long-term survival after endovascular aortic aneurysm repair (EVAR) in octogenarians remains unclear. We report, for the first time, that after EVAR, the life expectancy of octogenarians equals that of the matched octogenarians from the Rotterdam Study, provided that they do not develop early postoperative complications. Furthermore, if a complication occurred, octogenarians, had a nearly two-fold increase in long-term mortality compared to the general population octogenarians. Our results suggest that performing EVAR in octogenarians has a long-term beneficial impact on their life-expectancy, given that patients with low susceptibility to peri- and post-operative complications are selected.

Abstract

Background

Long-term survival after endovascular aortic aneurysm repair (EVAR) in octogenarians remains unclear. We evaluated this by comparing octogenarians after EVAR with a matched group of octogenarians without an abdominal aortic aneurysm (AAA) from the Rotterdam Study (RS). We also studied the influence of complications after EVAR on survival and aimed to identify risk factors for the development of complications in octogenarians.

Methods

Using propensity score matching (PSM), we matched 83 EVAR octogenarians on comorbidities with 83 octogenarians from the RS and compared survival between these two groups using Cox proportional hazard analysis. Then, we studied complications, defined as cardiac or pulmonary, renal deterioration, access site bleeding, acute limb ischemia or bowel ischemia, within 30 days of surgery between 83 EVAR octogenarians and 475 EVAR nonoctogenarians. Also, we studied the difference in baseline characteristics between the octogenarians with and without complications after EVAR and compared survival between the RS controls and the complicated and uncomplicated EVAR octogenarians separately.

Results

The total EVAR octogenarian population did not show an increased mortality risk compared to RS octogenarian controls (hazard ratio (HR) 1.28, 95% confidence interval (CI): 0.84- 1.97). Postoperative complications occurred in 22 octogenarians (27%) and 59 nonoctogenarians (12.4%, $p < 0.0001$), mainly due to the occurrence of cardiac (15.7% versus 4.9%, $p<0.0001$), pulmonary (4.8% versus 1.3%, $p=0.03$) and bleeding (6.0% versus 1.9%, $p=0.03$) complications. All baseline characteristics were similar in the complicated EVAR octogenarians compared to the uncomplicated EVAR octogenarians. After uncomplicated EVAR, octogenarians had a similar survival compared to the RS controls (HR 1.09, 95% CI: 0.68-1.77), but after complicated EVAR their mortality risk increased significantly (HR 1.93, 95% CI: 1.06- 3.54).

Conclusions

After standard EVAR, the life expectancy of octogenarians equals that of a matched group from the general population without an AAA, provided that they do not develop early postoperative complications. Patient selection and meticulous peri-operative care is key.

Keywords: Octogenarians, non-octogenarians, EVAR, complications, long-term survival

Introduction

With aging of the population, there has been an increase in octogenarians considered for endovascular aortic aneurysm repair (EVAR).(1) The technical success rate of EVAR in octogenarians, is high, although the complication rate was found to be higher compared with younger patients.(1, 2) Short-term benefits of AAA repair are clear (i.e. avoiding aneurysm rupture), but the question remains whether this preventive surgery will actually increase survival in octogenarians.

So far, studies have merely compared survival after EVAR between octogenarians and younger patients(2), the latter obviously having a higher life expectancy and usually less comorbidities.(1, 3, 4) A meaningful comparison of efficacy of EVAR among octogenarians should include control groups of the same age and risk profile. In the absence of any randomized controlled trials (RCTs), available data from large population-based cohort studies could be useful to compare long-term survival of octogenarians undergoing EVAR with matched controls.

Our main aim was to compare long-term survival in octogenarians after EVAR with matched octogenarian controls from the prospective population-based Rotterdam study (RS), without an AAA, using propensity score matching (PSM). Furthermore, we studied complications after EVAR. First, we compared post-EVAR complications between octogenarians and younger patients. Next, we sought to study the impact of complications on long-term survival after EVAR among octogenarians and to identify high-risk octogenarians for the development of these complications.

Methods

Study population

EVAR patient population

We used data of AAA patients treated with EVAR between January 2000 and December 2015 from a prospectively kept database at the Vascular Surgery Department of the Erasmus University Medical Center, Rotterdam, the Netherlands.

We excluded patients with ruptured aortic aneurysms and re-interventions after previous EVAR, as well as patients with a diagnosis other than degenerative AAA and isolated iliac aneurysms. All AAA's were infrarenal and treated with standard EVAR. We divided the patients into 2 groups; patients ≥ 80 years (octogenarians) and < 80 years of age (nonoctogenarians). A team of vascular anaesthesiologists preoperatively examined patients using the risk calculator of the ACS NSQIP®. If the perioperative risk was too high, patients were declined EVAR. These patients were not registered in the database. Informed consent was waived according to institutional policy on retrospective research. This study was approved by the Medical Ethics Committee (MEC-2019-0143).

Control group from the population-based Rotterdam Study

Controls were selected from Rotterdam Study (RS)[5], a prospective population-based cohort study that included participants aged 45 years or older in the district of Ommoord, in Rotterdam. The RS cohort began in 1990 with 7.983 participants. The cohort has been extended twice; in 2000 with 3.014 new participants and in 2006 with 3.932 new participants. The follow-up examinations take place every 3-4 years consisting of a home interview and two visits to the research center. An ultrasound of the abdomen measuring the diameter of the abdominal aorta is part of the visits. Participants are continuously monitored for major outcomes through access to general practitioners and municipality records. For the current study, we chose controls from visits of the RS that took place between April 2002 and March 2016 to have a similar recruitment period as that of the EVAR patient population. If the diameter of the aorta on ultrasound was greater than 30 mm, they were excluded as controls for this study. The RS was approved by the Medical Ethics Committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study). All participants provided written informed consent.

Clinical variables

Demographic characteristics of participants (age and sex), and comorbidities (ever smoking, hypertension, peripheral artery disease (PAD), ischemic heart disease (IHD), stroke, diabetes mellitus (DM) and cancer) were included in our analyses. Medical history and use of medication were obtained. Hypertension was defined as SBP >140 mm Hg, DBP >90 mm Hg, use of blood pressure-lowering medication, or a previous diagnosis of hypertension. Information about PAD and

cancer was obtained through medical interview and confirmed by checking general practitioners records for the RS and obtained from the medical history for the EVAR database. PAD was always symptomatic, and not solely based on an ankle brachial index. IHD was defined as myocardial infarction (MI), coronary artery bypass graft or percutaneous transluminal angioplasty, and was confirmed by ECG or medical records. Strokes identified in medical records were reviewed by research physicians, and verified by an experienced stroke neurologist for the RS and obtained from the medical history for the EVAR database. DM was defined as fasting glucose >6.9 mmol/L, nonfasting glucose >11.0 mmol/L, use of blood glucose-lowering medication, or a previous diagnosis of DM. Complications of interest after EVAR included cardiac complications, renal deterioration (defined as an increase in creatinine of > 0.5mg/dL or new hemodialysis), pulmonary complications, access site bleeding (any bleeding requiring blood transfusion or re-operation), acute limb ischemia and bowel ischemia intra-operatively and/or within 30 days postoperatively. Cardiac complications included atrial fibrillation, heart failure, myocardial infarction and/or elevated troponin levels. As a standard of care, after every vascular procedure, we measure serum level troponin 3 times a week. A postoperative hsTnT of 20 to 65 ng/L with an absolute change of 5 ng/L, a hsTnT >65 ng/L or a Trop T level > 0.03 were scored as elevated.(6, 7)

Clinical success of EVAR was defined as successful deployment of the endovascular device at the intended location without death as a result of aneurysm-related treatment, type I or III endoleak, graft infection or thrombosis, aneurysm expansion (diameter 5 mm, or volume 5%), aneurysm rupture, or conversion to open repair. The presence of graft dilatation of 20% or more by diameter, graft migration, or a failure of device integrity classified a case as a clinical failure.(8)

Outcomes and follow-up

EVAR patients were routinely followed with a 30-day and yearly computed tomography angiography (CTA). In selected patients with an anticipated lower risk of complications or renal function impairment, CTA was replaced by colored-duplex ultrasound or by non-contrasted CT. If an endoleak or sac growth were detected, patients underwent a CTA. The database of the Dutch Central Bureau of Statistics (CBS) was used to check mortality for the whole database as of April 26, 2016. Medical data on the study participants was anonymized by authorized data managers employed by the CBS. This data set was subsequently imported and linked to the Dutch death registry. According to Dutch privacy legislation,

data analysis was only allowed to an authorized researcher (KU) inside a secure environment after approval from the institutional ethical committee. We evaluated all-cause mortality. Information on the vital status of RS participants was obtained until June 13, 2016 from the central registry of the municipality in Rotterdam and through digital linkage with records from general practitioners. The completeness of follow-up on mortality within this cohort per common end date April 26, 2016, was 100 % as all participants (both from the RS and the EVAR database) were checked for mortality.(9)

Statistical analysis

Continuous variables are presented as mean (± standard deviation [SD]) and categorical variables as count (percentages, %). For the analyses shown in Table 2 and 3, continuous variables were compared with the Student t-test for variables with normal distribution and with the Mann-Whitney U Test for non-normally distributed variables. Categorical variables were compared using the Pearson's $\chi 2$ test. Analyses were performed on available cases per analysis. Missings were 0.6% for stroke, 1.3% for smoking, 1.4% for IHD, 3.7% for hypertension, 5.5% for clinical success, 8.4% for DM, 9.4% for cancer, 15% for PAD and 18% for serum troponin levels. We followed the STROBE guidelines (http://www.strobe-statement.org).

To properly select octogenarian controls from the RS cohort, we used Propensity Score Matching (PSM) with Greedy approach.(10) Several variables including age (at the moment of surgery for the EVAR patients, at the clinical visit for the RS controls), sex, ever smoking, and presence of comorbidities (hypertension, PAD, IHD, stroke, DM and cancer) were used to calculate the propensity scores. PSM was based on a logistic regression model that included EVAR intervention versus no intervention as a dichotomous outcome. PSM allows to compare every treated subject to every untreated subject and finds the closest possible match (Greedy approach). Closest pair will be paired off. This procedure will continue until there are no more possible pairings. Caliper suggested for the Greedy approach was <0.1. Plots were made to evaluate the distribution of propensity scores between the intervention and control groups. Through evaluating standardized differences, balance between intervention and control group was made after matching.

Further, we compared survival among EVAR octogenarians with survival of RS controls. Survival analysis was performed using Cox proportional hazards analysis. Time to all-cause mortality was the outcome of interest. All participants were followed-up until their date of death or censoring. Moreover, EVAR

octogenarians were divided into patients with and without 30-day postoperative complications. Kaplan Meier curves were built to compare total mortality among complicated, uncomplicated EVAR patients and matched controls. Cox proportional hazard model and parametric survival models . In the Cox proportional hazard model for time to primary outcome, a dummy variable of complications (0= RS controls, 1= EVAR uncomplicated, 2= EVAR complicated) was included. Cox proportional hazards assumptions were evaluated through Schoenfeld residuals, goodness of fit through linktest and Cox Snell residuals, linearity of covariates through martingales and devianza residuals and influential observations through dfbetas and cook distance. Akaike information criteria was used to select the best model. Statistical analyses was performed in STATA version 14.2 (Station College, Texas USA). All tests were two-sided and significance level was set to p-value <0.05.

Results

EVAR octogenarians versus Rotterdam Study octogenarians

83 octogenarians underwent EVAR (mean age 83.0±2.9 years). For PSM, 2212 octogenarian controls from the RS were initially included (mean age 83.5±2.9 years). Table 1 details the characteristics of octogenarians before and after PSM. The 83 RS controls matched the 83 EVAR cases after PSM. Figure 1 depicts the plot distribution of propensity scores before and after matching. The distribution of log odds propensity scores between the two groups was identical after PSM. We first examined the survival of the 83 EVAR octogenarians with the 83 RS controls based on PSM and found no difference in survival (HR 1.28, 95% confidence interval (CI) 0.84- 1.97) (Figure 2-A).

EVAR octogenarians versus EVAR nonoctogenarians

Table 2 shows the baseline characteristics of EVAR octogenarians and nonoctogenarians, as well as the complications after EVAR. Mean age was 83.0±2.9 for the octogenarians and 70.2±6.3 years for the nonoctogenarians. Although non-significant, there were more women in the octogenarian group (16.9% versus 9.7%, p= 0.052) with a slightly larger median aneurysm diameter (63.5 mm versus 60.5 mm, p=0.053) compared with the nonoctogenarians. Complications occurred in 22 octogenarians (27%) and in 59 nonoctogenarians (12.5%) (p <0.0001), mainly due to the occurrence of cardiac complications (15.7% versus

4.9%, p<0.0001, of which 3.6% asymptomatic troponin increase in octogenarians and 2.5% in nonoctogenarians, ns). Respiratory (4.8 % versus 1.3%, p=0.03) and bleeding complications (6.0% versus 1.9%, p=0.03) also occurred more often in octogenarians (Table 2). None of the patients who had a bleeding complication also had a cardiac complication. There was no difference between the groups with respect to type of anaesthesia.

Uncomplicated versus complicated EVAR octogenarians

We further stratified the EVAR octogenarians to uncomplicated (n=61) and complicated EVAR (n=22). When comparing baseline characteristics between the two groups (Table 3), there was no statistic significant differences.

Compared with the matched octogenarians from the RS, octogenarians that underwent EVAR without any complications, did not have an increased risk of mortality (HR 1.09, 95% CI 0.68-1.77). After 6 years of follow-up, 50% of uncomplicated EVAR octogenarians were still alive. However, octogenarians with complications after EVAR, had an increased risk of mortality compared to controls from the RS (HR 1.93, 95% CI 1.06- 3.54) (Figure 2-B).

Discussion

The main finding of our study was that octogenarians who underwent EVAR had the same long-term survival as matched octogenarians from the general population without an AAA. This suggests that in this group the presence of aneurysm disease by itself, did not negatively influence overall survival. Six years after EVAR, 50% of our uncomplicated EVAR octogenarian patients were still alive. The powerful approach of PSM enabled us to create a matched control group of octogenarians, without an AAA, from the general population. To our knowledge, this is the first study in which survival of octogenarians after EVAR versus a matched cohort of octogenarians is described. We report for the first time, that octogenarians after complicated EVAR had a nearly two-fold increase in mortality compared to the general population of octogenarians. Thus, pre-operative patient selection is key. As long as we cannot identify these high risk patients, our findings raises the question if we should adapt the threshold for AAA repair with increasing age.

The baseline characteristics in our study, including smoking, hypertension, PAD, stroke and IHD, did not differ between octogenarians and younger patients, which

differs from the findings of other EVAR studies.(1, 11, 12) In our institution, a team of vascular anaesthesiologists preoperatively examine all patients using the surgical risk calculator of the ACS NSQIP®. Treatable comorbidities are corrected where possible in order to perform surgery in patients with a good physical condition. If the perioperative risk is too high, patients are declined from EVAR. This might, at least partly, explain the similarity in the prevalence of risk factors between the octogenarians and nonoctogenarians at baseline. This might also explain why the long-term survival after uncomplicated EVAR was similar to that of the matched octogenarians from the RS; we have selected the fittest patients for surgery. Unfortunately, the turndown rate was unknown so we were unable to compare the patients unfit for surgery with the controls from the RS. A previous study on outcome after ruptured AAA repair in octo- and nonagenarians also found that if patients survived the first 90 days, their long-term survival was only marginally decreased compared with age and sex- matched controls. In these very old cohorts, aneurysmal disease by itself seems to play a less important role in their survival.(13)

We showed that cardiac complications occurred more frequently among octogenarians compared with nonoctogenarians after EVAR and to a lesser extend pulmonary and bleeding complications. Considering that comorbidities of our two groups were similar at baseline, we hypothesise that the EVAR procedure triggered the occurrence of such complications. Cardiac complications in our study occurred more often than what has been reported in the literature(2, 14, 15) and several studies did not find a higher occurrence of MI after EVAR in octogenarians.(1, 12, 16) The larger cardiac complication rate in our study is explained by the fact that we used a combined endpoint of atrial fibrillation, heart failure, myocardial infarction and elevated troponin levels. We routinely measure postoperative troponin levels in all of our patients. Therefore, we were able to identify elevated troponin levels in patients without symptoms and counted these as cardiac complications.

A postoperative increase in troponin levels has been reported as a risk factor for early mortality (also in our EVAR patients).(7, 17) Checking this routinely might lead, at an early stage, to the identification of patients that need a cardiac intervention. At our institution, it is now also checked pre-operatively for all patients. This can be helpful in predicting which octogenarians will develop postoperative cardiac complications, as was recently reported.(18) In the current ESVS guidelines on management of AAA there is no recommendation on checking troponin levels routinely prior or after EVAR.(19) It is recommended that in patients with poor functional capacity or with significant clinical risk factors referral for cardiac work up and optimisation is recommended prior to elective abdominal aortic aneurysm

repair. More research is needed to confirm that in this elderly population a different cardiac work up might be needed compared with younger patients planned for EVAR.

To diminish pulmonary complications, performing the procedure under local anaesthesia might be considered, as significantly less pulmonary complications have been reported.(20) Lastly, a careful selection has to be made with respect to access strategy (open or percutaneously) in order to decrease the chances of bleeding complications. The guidelines recommend that an ultrasound guided percutaneous approach should be considered in EVAR. No recommendation is done on type of anaesthesia.(19) Further research is necessary to identify more risk factors that predict peri-operative complications. For the whole group of EVAR patients, patients without PAD less often had elevated troponin levels (3.5% versus 9.3%, p=0.03) and cardiac complications (5.5% versus 12.4%, p=0.02), compared to patients with PAD.

Current guidelines lack recommendations on treatment in relation to age of the patient or risk of complications. As EVAR is a preventive treatment, risks and benefits should be weighed. If the risk of complications is high, a higher AAA diameter threshold should be selected for treatment. Current guidelines only state that elective AAA repair is not recommended in patients with a limited life expectancy (2 to 3 years).(19)

Before EVAR, our octogenarians were at an increased risk of aneurysm rupture and death as they had large diameter AAAs. A surveillance study in patients unfit for AAA surgery showed that during a follow-up period of over 6 years 11% died of rupture when the AAA was 5.1-6.0 cm, 20% when it was 6.1-7.0 cm and 43% with an AAA > 7.0 cm.(21) The mean aneurysm diameter at the time of treatment was 63.5 mm for our octogenarians equalling a significant annual rupture risk. Although our study is not a RCT randomizing between EVAR and no treatment, survival curves of the octogenarians with an AAA would likely have been lower compared to the survival curves we now showed in Figure 2, if we had left the AAA untreated.

Strengths of our study include detailed characterization of a well-defined AAA patient group, use of PSM to define a matched control group from the general population, availability of detailed information on a variety of risk factors as well as long-term follow-up data for both AAA patients and the control group. The limitations of our study also merits attention. This was not a RCT and our control group is not the best to answer the question whether or not octogenarians have an increased survival after EVAR. For that we should have compared them with

octogenarians with an AAA who were not treated with EVAR. Unfortunately, we don't have these controls from the Rotterdam study as all octogenarians with an AAA had been referred to a vascular surgeon and we did not register which patients were turned down for EVAR. Also, we will never know if patients who developed complications could have had a better survival if their AAA was not treated. Although all patient data were carefully collected prospectively, there is still a chance that relevant characteristics might not have been recorded. Furthermore, this study was performed in a single tertiary referral institution and may therefore not be applicable to patients treated in other hospitals. Also, we only matched on comorbidities known for our patients so the matching will not have excluded all confounding variables (residual confusion). We did not match the complicated and uncomplicated EVAR octogenarians with 2 separate groups of RS controls. Lastly, our patient group was relatively small, especially when performing subgroup analyses. Therefore, we might have been underpowered to detect significant smaller differences between the groups.

Conclusions

This is the first study to compare octogenarians after EVAR with matched octogenarian controls without an AAA from the general population. The life expectancy is equal in both groups, provided that the octogenarians do not develop early postoperative complications. If complications do occur, their mortality risk nearly doubles. Future research should focus on developing algorithms to identify which octogenarians are prone to develop complications and to optimize peri-operative care to lower the chance of occurrence of complications around EVAR treatment.

Conflict of interest disclosures: None

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Table 1. Baseline characteristics before and after propensity score matching for EVAR octogenarians and the Rotterdam Study controls

	Before propensity score matching			**After propensity score matching**		
	EVAR (n=83)	**RS (n= 2212)**	**p-value**	**EVAR (n=83)**	**RS (n=83)**	**p-value**
Age, years	83.02 (3.0)	83.45 (2.9)	0.18	83.02 (3.0)	82.56 (2.5)	0.28
Female gender, n (%)	14 (16.9%)	1350 (61.0%)	<0.001*	14 (16.9%)	16 (19.3%)	0.69
Smoking (ever), n (%)	58 (69.9%)	773 (35.4%)	<0.001*	58 (69.9%)	60 (72.3%)	0.73
Hypertension, n (%)	65 (78.3%)	1811 (84.9%)	0.10	65 (78.3%)	66 (79.5%)	0.85
PAD, n(%)	11 (13.3%)	176 (9.2%)	0.21	11 (13.3%)	10 (12.1%)	0.82
IHD, n(%)	32 (38.6%)	314 (14.4%)	<0.001*	32 (38.6%)	32 (38.6%)	1.00
Stroke, n(%)	15 (18.1%)	177 (8.1%)	<0.01*	15 (18.1%)	14 (16.9%)	0.84
DM, n(%)	15 (18.1%)	393 (19.3%)	0.79	15 (18.1%)	19 (22.9%)	0.44
Cancer, n(%)	19 (22.9%)	280 (13.9%)	0.02*	19 (22.9%)	24 (28.9%)	0.38

* Statistically significant difference between the EVAR octogenarians and the Rotterdam Study octogenarian controls

Abbreviations: EVAR, endovascular aortic aneurysma repair; RS, Rotterdam Study; PAD, peripheral artery disease; IHD, ischemic heart disease; DM, diabetes mellitus

Table 2. Baseline characteristics and perioperative complications of EVAR octogenarians and nonoctogenarians

	Octogenarians N=83	Nonoctogenarians N=475	P-value
Age at surgery, years	83.0 ± 2.9	70.2 ± 6.3	<0.001*
Aneurysm diameter, mm	63.5 ± 10.4	60.5 ± 12.6	0.05
Female gender, n (%)	14 (16.9)	46 (9.7)	0.05
Smoking (ever), n (%)	55 (68.8)	353 (76.7)	0.12
Hypertension, n (%)	65 (78.3)	330 (70.8)	0.16
PAD, n (%)	11 (13.4)	78 (16.9)	0.44
IHD, n (%)	32 (38.6)	167 (35.8)	0.62
Stroke, n (%)	15 (18.1)	63 (13.6)	0.29
Pulmonary disease, n (%)	13 (16.9)	70 (15.4)	0.73
DM, n (%)	15 (18.1)	77 (16.5)	0.72
History of cancer, n (%)	13 (17.6)	91 (20.9)	0.51
Clinical success, n (%)	62 (74.7)	353 (74.3)	0.86
Complications < 30 days†	22 (27)	59 (12.5)	<0.0001*
Cardiac, n (%)	13 (15.7)	23 (4.9)	<0.0001*
Asymptomatic troponin increase, n (%)	3 (3.6)	12 (2.5)	0.37
Renal deterioration, n(%)	0	5 (1.1)	
Pulmonary, n(%)	4 (4.8)	6 (1.3)	0.03*
Access site bleeding, n(%)	5 (6.0)	9 (1.9)	0.03*
Limb ischemia, n(%)	1 (1.2)	12 (2.5)	0.46
Bowel ischemia, n(%)	1 (1.2)	0	

*Statistically significant difference between the octogenarians and nonoctogenarians. Abbreviations: PAD, peripheral artery disease; IHD, ischemic heart disease; DM, diabetes mellitus; MI, myocardial infarction. † Complications of interest occurred intra-operatively and/or within 30 days postoperatively.

Table 3. Baseline characteristics of uncomplicated and complicated EVAR octogenarians

	Uncomplicated EVAR octogenarian (n=61)	**Complicated EVAR octogenarian (n=22)**	**p-value**
Age at surgery, years	83.1 ± 2.9	82.7 ± 3.3	0.58
Aneurysm diameter, mm	64.3 ± 11.2	61.4 ± 7.8	0.28
Female gender, n (%)	10 (16.4)	4 (18.2)	0.85
Smoking (ever), n (%)	41 (67.2)	17 (77.3)	0.38
Hypertension, n (%)	45 (73.8)	20 (90.9)	0.10
PAD, n (%)	6 (9.84)	5 (22.7)	0.13
IHD, n (%)	23 (37.7)	9 (40.9)	0.79
Stroke, n (%)	8 (13.1)	7 (31.8)	0.05
Pulmonary disease, n (%)	8 (13.1)	5 (22.7)	0.29
DM, n (%)	10 (16.4)	5 (22.7)	0.51
History of cancer, n (%)	16 (26.2)	3 (13,6)	0.23

Abbreviations: PAD, peripheral artery disease; IHD, ischemic heart disease; DM, diabetes mellitus

Figure 1 Distribution of propensity scores for the EVAR octogenarians and matched octogenarian controls from the Rotterdam Study before and after propensity matching procedure.

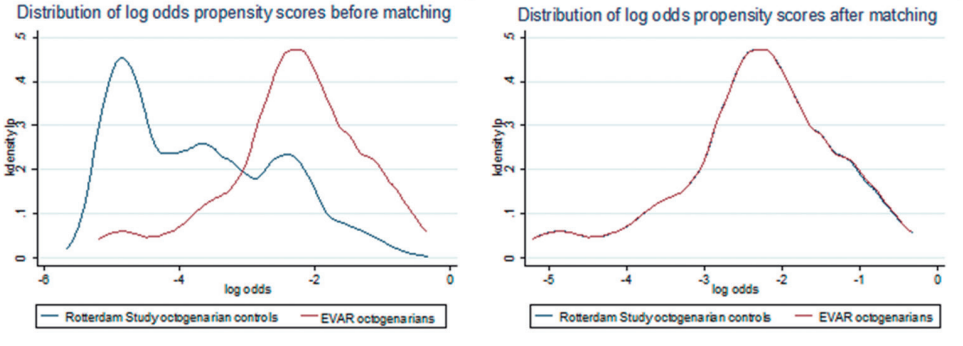


Figure 2A Proportion surviving between EVAR octogenarians and octogenarians from The Rotterdam study cohort.

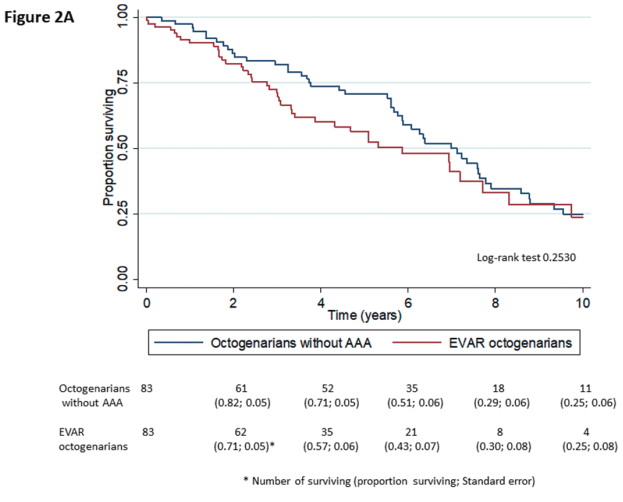
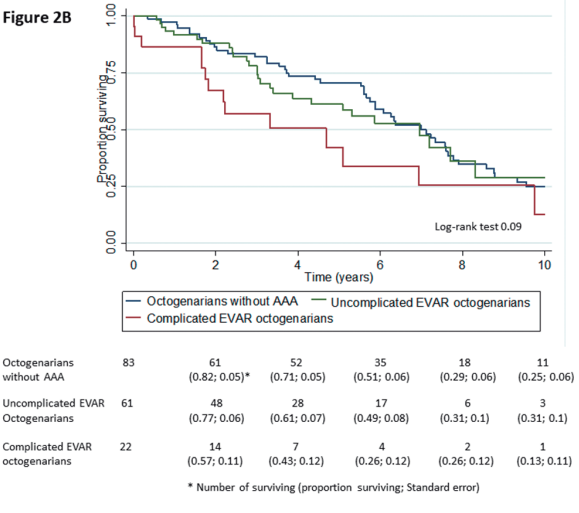


Figure 2B Proportion surviving between Uncomplicated EVAR octogenarians, complicated EVAR octogenarians and octogenarians from The Rotterdam study cohort.

3A.1



CHAPTER 3A.2

Ten-year survival after FFR-guided strategy in patients with an isolated proximal left anterior descending coronary stenosis.

Poster Presented in European Society of Cardiology Congress 2018:

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doi: 10.1016/j.jacc.2019.07.013

Abstract

Aim. To investigate 10-year survival of patients with an isolated stenosis in the proximal left anterior descending coronary artery (LAD) in whom the treatment strategy was based on Fractional Flow Reserve (FFR).

Methods. In 729 patients with an intermediate stenosis in the proximal LAD and in whom treatment strategy was based on FFR measurements, the rates of all-cause death, myocardial infarction (MI), and target vessel revascularization (TVR) were followed up during 10 years. When FFR was >0.80, medical therapy was chosen (MT group, n=564). When FFR was ≤0.80, revascularization therapy was performed (REV group, n= 165). All-cause mortality of both groups was compared with two corresponding control groups without known CAD at baseline from the population-based Rotterdam Study using Propensity Score Matching Greedy approach.

Results. The follow-up was complete for all-cause mortality. At 10-year, the MT and REV groups did not differ significantly for all-cause mortality (hazard ratio[HR] 0.97; 95% Confidence Interval[CI]: 0.67 to 1.40). In contrast, when compared to their respective control groups, all-cause mortality was significantly higher in the MT group (HR: 1.50; 95% CI: 1.14 to 1.96) and in the REV group (HR 2.22; 95% CI: 1.28 to 3.86).

Conclusions. In patients with an isolated stenosis in the proximal LAD, medical therapy for FFR-negative stenosis and revascularization of FFR-positive stenosis are associated with similar survival rates. Yet, regardless of treatment strategy, patients with an isolated LAD stenosis have a significantly higher all-cause death than their matched controls without known CAD at baseline.

condensed abstract

Ten year clinical follow-up was obtained in 729 patients with an isolated stenosis in the proximal left anterior descending coronary artery and in whom the decision

about revascularization had been taken based on FFR measurements. Patients in whom revascularization was deferred (FFR>0.80) showed a similar mortality as patients who underwent revascularization (FFR ≤0.80). In contrast, both groups had a higher all cause mortality than their respective matched controls from the general population. This suggests that the presence of atherosclerosis in addition to ischemia, is a major determinant of outcomes.

KEYWORDS: FFR, 10-year follow-up, healthy controls, propensity score matching.

Abbreviations:

CABG: Coronary Artery By-pass Grafting

$\mathbf{C_{MT}}$: Rotterdam matched controls for Aalst Medical Therapy group;

$\mathbf{C_{Revasc}}$: Rotterdam matched controls for Aalst Revascularization group;

FFR: Fractional Flow Reserve;

LAD: Left Anterior Descending;

MACE: Major Adverse Cardiovascular Events;

MI: Myocardial Infarction;

MT: Aalst Medical Therapy Group;

REV: Aalst Revascularization Group;

TVR: Target Vessel Revascularization;

Introduction

The presence of a stenosis in the proximal left anterior descending coronary artery (LAD) is considered to bear a poor prognosis and this resulted in specific guideline recommendations[1,2]. Recent European guidelines recommend revascularization in patients presenting with an angiographic >50% diameter stenosis in the proximal LAD[3,4]. However, the predictive value of coronary angiograph to determine the

hemodynamic significance of a stenosis is low[5,6]. In contrast, Fractional Flow Reserve (FFR) is considered the standard of reference to assess coronary stenosis severity and to guide therapy[5,7-11].

Accordingly, the aim of the present study was twofold:

First, we assessed the effects of FFR guidance on long-term outcomes of patients with an isolated and "intermediate" proximal LAD stenosis on coronary angiography. Therefore we compared outcomes of patients with FFR-negative stenosis who were medically treated with those of patients in whom the stenosis was FFR-positive and subsequent revascularization was performed.

Second, we examined the impact of the presence of coronary atherosclerosis on all-cause mortality by comparing the 10-year survival of the two patient groups with those of community-based control groups from the Rotterdam Study cohort based on propensity matching approach, using the Greedy algorithm.

Methods

Study design and participants

Intervention groups

The design and methods of the study have been previously described and are briefly summarized below[12]. We examined 6107 patients admitted between October 1999 and November 2008 for chest pain and in whom a coronary angiogram and FFR measurements were performed. For the sake of the present analysis we included only patients in whom an isolated 30% to 70% (visual estimate) LAD stenosis was present (N=729). Patients with a stenosis in another coronary segment, or with concomitant valvular pathology were excluded.

Stenosis severity was assessed in at least 2 orthogonal views for best imaging of the proximal LAD lesion. Patients where the Proximal LAD stenosis was evaluated by FFR measurements were included in the present analysis. FFR measurements were performed with a pressure wire (Radi Medical, Uppsala, Sweden). Hyperemia was induced by either intravenous (140 μg/kg/min) or intracoronary adenosine as previously described[12]. Patients with an FFR value >0.80 (FFR-negative stenosis) were treated by medical therapy (MT group, n=564). In patients with an FFR value ≤0.80 a revascularization was performed (REV group, n=165). The local database was used to retrieve demographic and baseline clinical data.

All-cause mortality was defined as the total number of cardiovascular, non-cardiovascular and undetermined deaths observed during the follow up period[13]. Myocardial infarction(MI) was defined according to the third universal definition as the detection of a rise and/or fall of cardiac troponin (cTn) with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following: Symptoms of ischemia new or presumed new significant ST-segment–T wave (ST–T) changes or new left bundle branch block (LBBB), development of pathological Q waves in the ECG, imaging evidence of new loss of viable myocardium or new regional wall motion abnormality, identification of an intracoronary thrombus by angiography[14]. Target vessel revascularization was defined as any new revascularization procedure including at least the LAD 5-mm proximal and distal edge to the stent/scaffold in the REV group or the initial lesion in the MT group. Major Adverse Cardiovascular Event was defined as the occurrence of at least one of the above described events (Death, Myocardial Infarction or Target Vessel Revascularization).

Follow-up of the patients was obtained by a combination of (a) direct access to the Belgian Population Registry, (b) the electronic medical file and local data base, (c) telephone contact, and (d) written questionnaires. When the patients reported any type of event which was not documented in the local medical file or the local database, the clinical details about this event were obtained by a dedicated nurse. The patients were included in the study between January 2000 and November 2008. Follow up period lasted 10 years after the index procedure.

Control group

Control groups were selected from 8451 participants free of CHD at baseline from the prospective population-based Rotterdam Study (RS). The RS cohort has been described in detail[15]. The first RS cohort (RS-I) started in 1990 and included 7983 persons of 55 years or older living in the well-defined Ommoord district in the city of Rotterdam, The Netherlands. The cohort has been extended twice (in 2000, RS-II, 3011 participants; in 2006, RS-III, 3932 participants) to include individuals who had become 55 years of age or moved into the study district since the start of the study. All RS participants have been re-examined every 2-3 years. The current analyses included participants who visited the RS research center for their regular follow-up visits between January 2000 and November 2008 (RS-I-4, RS-II-1, RS-II-2, and RS-III-1), similar to the recruitment window of the intervention group. The Rotterdam Study has been approved by the Medical Ethics Review Board of Erasmus Medical Center and by the

Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.

To ascertain death and cause of death for all RS participants, mortality data were obtained through the central registry of the Municipality of Rotterdam, records from collaborating general practitioners, and information from follow-up visits. The Central Registry of Genealogy of The Netherlands was consulted when the vital status of participants was missing. Complete mortality follow-up was available until August 1, 2016.

Statistical analysis

Continuous variables were presented as mean (standard deviation) and categorical variables as percentages. Between the two treatment groups, continuous variables were compared by independent samples t tests and dichotomous variables by Fisher exact or chi-square tests, as appropriate. For matching the samples, continuous variables were compared with the student t-test paired for variables with normal distribution and with Wilcoxon test for non-normally distributed variables. Categorical variables were compared using McNemar test.

Control group selection

Control group selection from RS cohort was performed independently for each of the intervention groups (MT and REV groups) based on the propensity score matching (PSM) approach, Greedy algorithm. Several variables including age, sex, dyslipidemia, body mass index (BMI), smoking, hypertension, and family history of cardiovascular disease were used to calculate propensity scores. To calculate propensity scores, a logistic regression model was developed that included intervention (versus non-intervention) as a dichotomous outcome. PSM Greedy algorithm allows comparisons between every treated subject to every untreated subject and finds the closest match. This is an iterative process. Closest pair will be paired off. This procedure will continue until there are no more possible pairings. For the PSM Greedy approach, the suggested caliper was <0.1 standard deviations. Non-linear terms for age and BMI and the interaction terms between sex and smoking were included in the models. Plots to evaluate the distribution of propensity scores between intervention and control groups were made. Balance

between intervention and control groups was made after matching, evaluating standardized differences.

Kaplan Meier curves were built to compare all-cause mortality (primary outcome) between the two intervention groups (MT and REV) and between each of the intervention groups (MT and REV) and their respective control groups. A Cox proportional hazard model for time to primary outcome was developed, including only intervention versus non-intervention in the model with completely balanced covariates (PSM Greedy) and adjusted for covariates in case of imbalance. For each of the final models, Cox proportional hazards assumptions were evaluated through Schoenfeld residuals, goodness of fit through linktest and Cox Snell residuals, linearity of covariates through martingales and devianza residuals and influential observations through dfbetas and cook distance. Statistical analyses were performed in STATA version 14.2 (Station College, Texas USA). The level of significance was denoted as p-value ≤0.05.

Results

FFR-guided Medical Therapy versus Revascularization

Among 729 consecutive patients with an isolated proximal LAD stenosis, 564 had an FFR> 0.80 and received medical therapy (MT group), and 165 had an FFR ≤ 0.80 and underwent revascularization (REV group). In the revascularization group 13% had CABG (all IMA) and 87% had PCI .The baseline characteristics of the MT and REV groups are given in **Table 1**. The follow-up for mortality was complete for both groups while follow up for MI or TVR was 90% and 98% complete in the MT and REV groups respectively. The mean follow-up for survival free of major adverse cardiac events was 97.01±33.7 months for MT and 81.4±43.5 months for REV group respectively.

The outcome data of the MT and REV groups are given in **Figure 1** and in **Table 2**. There was no difference in all-cause mortality at 10 years, between patients in the MT group vs patients in the REV group (75% vs 75% respectively; hazard ratio [HR]: 0.96; 95% confidence interval [CI]: 0.67 to 1.38; p=0.85) (**Figure 1A**).

The survival free of death or MI estimates at 10 years were higher in patients of the MT group than in patients in the REV group (77% versus 68%, respectively; HR (95% CI): 0.65 (0.43 to 0.91; p=0.001) (**Figure 1B**).

The MACE-free survival estimates at 10 years were higher in patients of the MT group than in patients in the REV group. (68% versus 53%, respectively; HR (95% CI): 0.56 (0.37 to 0.71; p<0.0001) (**Figure 1C**). The details of the cardiac events observed in the MT and REV groups are given in **Table 2**.

Patient Groups versus Rotterdam Study Controls

For each patient group (MT and REV), a control group (1:1) from an initial pool of 8451 participants of the RS cohort was chosen by PSM Greedy algorithm. The very close match between each patient group and its respective control group is illustrated by the Lowess graph. (**Figure 3, 4)**

All-cause mortality for patients who received medical therapy (MT group) was significantly higher than that of their respective RS controls; HR: (95% CI): 1.50 (1.14 to 1.96). Likewise, all-cause mortality for patients among whom a revascularization was performed (REV group) was significantly higher than that of their respective RS controls; HR (95%CI): 2.22 (1.28 to 3.86). (**Figure 2 A and B**)

Discussion

Summary of findings

The present analyses based on long term follow-up of patients with an isolated stenosis in the proximal LAD at conventional angiography indicates that the 10-year survival was similar in patients in whom revascularization was deferred versus those in whom revascularization was performed based on an FFR- guided strategy although the rate of MI and TVR was lower in medically treated patients. In contrast, when the MT and REV groups were compared with closely matched community-based controls from the Rotterdam Study, a significantly higher all-cause mortality was observed in both patients groups as compared to their respective control groups. This indicates that in patients with a proximal LAD stenosis, survival is similar when ischemia producing lesions are alleviated, but that patients with documented coronary atherosclerosis have a significantly higher mortality at 10 years than a population without known coronary artery disease at baseline but with the same risk profile. Taken together these findings suggest that the presence of coronary atherosclerosis is a major determinant of survival, more than the presence of ischemia.

Previous studies on patients with isolated proximal LAD stenosis

An isolated narrowing in the proximal LAD is sometimes referred to as the "widow maker" as it is purportedly associated with a high mortality rate. Yet, the present data extend to isolated proximal LAD stenosis the overall favorable long-term mortality estimates associated to medical therapy as reported in previous trials when the stenosis is hemodynamically non-significant[6,9,10]. A true comparison with other studies is difficult due to the small number of patients, the short follow-up period, or the markedly younger age of the patients[16-18]. In addition, in most of them FFR was not measured and all patients underwent revascularization on the basis of the angiogram. Recently, Kjoller-Hansen and colleagues reported a 25% 10-year all cause mortality in 365 patients with an isolated proximal LAD treated with a drug eluting stent[19]. This compared favorably with a 26.3% of mortality in the 1114 patients with a non-proximal LAD stenosis. This mortality rate is also in the same range as in a recently reported large registry data[20], where an overall mortality of approximately 8% was found 3 years after revascularization by either PCI or CABG. This suggests that, when ischemia is absent – in case of FFR >0.80 - or when ischemia is alleviated by revascularization, survival rates are similar. Conversely, it is therefore very unlikely that in patients with a hemodynamically non-significant stenosis, a revascularization procedure would have improved survival.

In the present study, patients with an isolated hemodynamically non-significant stenosis in the proximal LAD had an approximately 30% estimated mortality rate at 10 years, which is significantly higher than the mortality rate observed in controls closely matched for their risk profile. This difference persists even after revascularization.

Mechanisms of the 'protective role' of a high FFR.

In hemodynamically non-significant stenosis, the rate of spontaneous MI's was particularly low and half of them occurred in other vascular territories. A similar observation had already been made in the FAME trial. Among the 513 lesions deferred on the basis of FFR measurements only a small minority led to a MI related with the index lesion[7]. This suggests that a high FFR identifies lesions at low risk not only because they are not associated with stress-induced reversible ischemia but also because their rate of progression is low. The underlying mechanism of this relationship is elusive. Yet, it strongly suggests that the physical forces (mainly plaque stress, shear stress, intra-plaque pressures) related to the presence of a

pressure gradient do impact the progression of a stenosis. The corollary is that when FFR is high, there is no pressure gradient and these physical forces are absent and thus lesion progression is low and increase its potential to rupture. The present data confirm the low risk of plaque destabilization and is at odds with the general belief that acute coronary occlusions mainly occur at the level of mild stenosis.

In the present study we included stenosis between 30 and 70%. These lesions can indeed be associated with low FFR values even when the angiographic aspect is very mild and when the resting hemodynamics is almost normal ("pseudo-mild stenosis")[21]. After the LM, the proximal LAD is the arterial segment that supplies the largest myocardial mass. In these territories, the increase in blood flow after administration of a hyperemic agent can be very important and the increase in gradient larger than expected. Accordingly, in these prognostically important segments, it is advisable to keep the threshold as low as possible for physiologic assessment and to resist the temptation to avoid hyperemia[22].

Limitations

A number of limitations have to be taken into account.

First, the study patients were not randomized. Rather, their therapy was guided by the FFR value of the proximal LAD stenosis. In addition, events were not adjudicated and the analysis was under-powered for mortality comparison. Yet, in this registry, the degree of completeness of 10-year follow-up is higher than in many controlled randomized trial with a much shorter follow-up. The vast majority of patients were followed-up at the outpatient clinic of the Center, their data centralized in the local database and controlled by dedicated research nurses.

Second, selection of the patients was based on the visual estimation of the proximal LAD stenosis as well as on the clinical context: "typicality" of the complaints, presence of a previous MI, presence of non-invasively assessed ischemia, risk of the revascularization procedure, global LV function, among others. While this reflects daily clinical practice, this leaves room for some subjectivity. One should therefore bear in mind that the results cannot be extrapolated to patients with angiographically tight stenosis.

Third, the vast majority of patients in the MT group were stable (94.2%). The conclusions of the study can therefore not be extrapolated to patients with acute coronary syndromes. While waiting for the results of prospective trials in acute

coronary syndromes, it should be reminded that FFR measurements are not routinely recommended in potentially culprit stenoses of acute coronary syndromes.

Finally, in the Rotterdam Study, only individuals older than 55 years old have been included. Subsequently, we restricted our analysis to patients older than 55 years. This increase in mean age of our study population may partially explain the somewhat higher rate of all cause mortality in the present study as compared to the analysis recently presented by Kjoller-Hansen et al[19].

Conclusion

These long-term clinical outcome data confirm that patients with a hemodynamically non-significant stenosis in the proximal LAD have a favourable 10-year survival, even though no revascularisation procedure had been performed initially. These patients have a similar survival rate as patients with a significant stenosis in the proximal LAD who underwent revascularization. However, their risk of all-cause mortality is higher as compared with a general population without documented coronary atherosclerosis at baseline. This suggests that the presence of atherosclerosis, in addition to ischemia, is a major determinant of outcomes.

CLINICAL PERSPECTIVES

Isolated stenosis in the proximal left anterior descending coronary artery (LAD) is sometimes referred to as the 'widow maker' to allude to its poor prognosis. The present data indicate that – as for all other coronary segments – FFR guided decision making about revascularization is safe and effective.

When comparing these patients with matched controls without known coronary artery disease at baseline, a significant difference in mortality was observed. This difference continued to accrue over time. This difference was observed in FFR-negative patients who did not undergo revascularization but also in FFR-positive patients who underwent revascularization. These data indicate that in patients with an angiographically intermediate proximal LAD stenosis survival is determined by the presence of atherosclerosis more than by the presence or absence of ischemia.

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Legend of Figures

Figure 1

Kaplan-Meier Curves for the Medical and the Revascularization Groups.

Shown are percent survival (Panel A), percent survival free from death or myocardial infarction (Panel B), and survival free of major adverse cardiac events (death, myocardial infarction, or target vessel revascularization) (Panel C).

Figure 2

Panel A: Kaplan-Meier Curves for All-Cause Mortality for the Medical Treatment Group versus Rotterdam Study Controls

Panel B: Kaplan-Meier Curves for All-Cause Mortality for the Revascularization Group versus Rotterdam Study Controls

Figure 3

Panel A: Distribution of Log Odds of Propensity Scores using Greedy Algorithm

for Selection of Control Group for the Medical Intervention Group

Panel B: Distribution of Log Odds of Propensity Scores using Greedy Algorithm for Selection of Control Group for the Revascularization Group

Central illustration

Kaplan-Meier Curves for All-Cause Mortality for the Medical Treatment and Revascularization Groups versus their respective controls (broken lines). The insert show a typical example of a stenosis in the proximal left anterior descending coronary artery. Were the FFR be higher than 0.80, this patient would have been treated medically; Were the FFR be lower equal than 0.80, this patient would have been revascularized.

Figures

Figure 1A

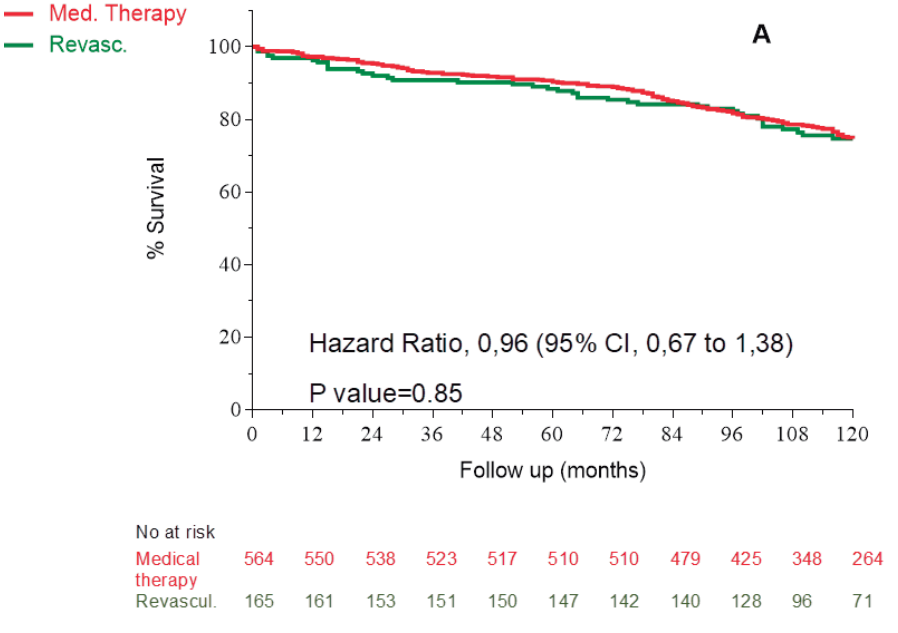
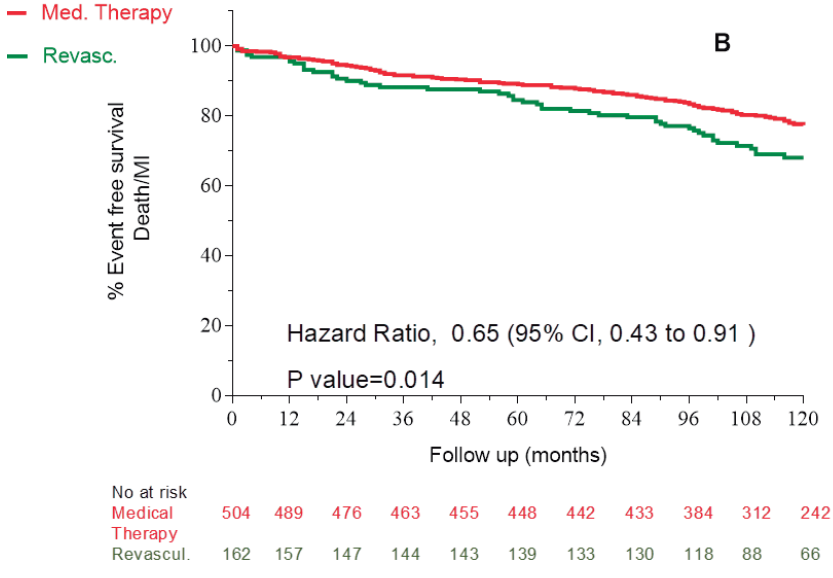


Figure 1B


Med. Therapy
Revasc.
B
% Event free survival
Death/MI
100
80
60
40
20
0
Hazard Ratio, 0.65 (95% CI, 0.43 to 0.91)
P value=0.014
0 12 24 36 48 60 72 84 96 108 120
Follow up (months)
No at risk
Medical Therapy 504 489 476 463 455 448 442 433 384 312 242
Revascul. 162 157 147 144 143 139 133 130 118 88 66

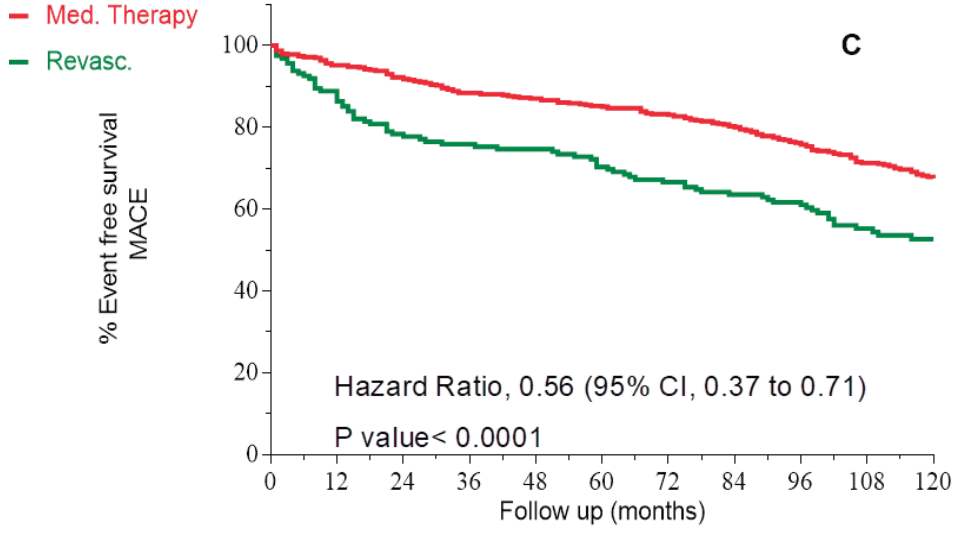
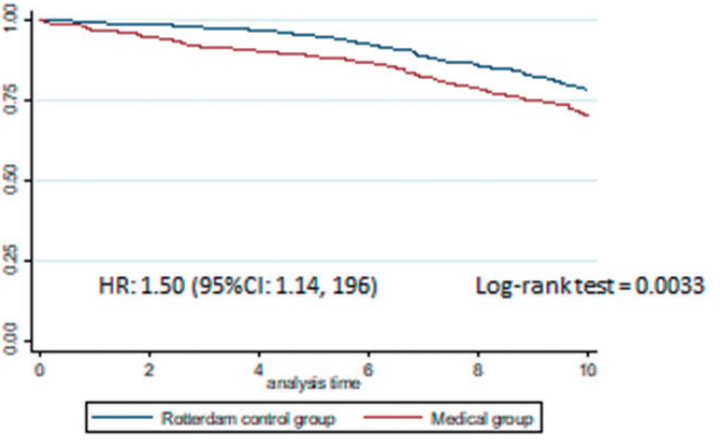
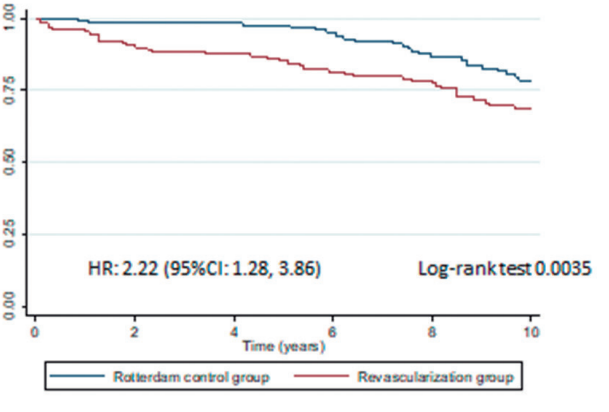

Med. Therapy
Revasc.
C
% Event free survival
MACE
100
80
60
40
20
0
Hazard Ratio, 0.56 (95% CI, 0.37 to 0.71)
P value< 0.0001
0 12 24 36 48 60 72 84 96 108 120
Follow up (months)
No at risk
Medical Therapy 504 482 464 447 438 428 418 403 349 279 214
Revascul. 162 145 127 124 122 117 109 104 95 68 48

Figure 2A



	0	1	2	3	4	5	6	7	8	9	10
Medical	461	447	437	421	415	409	400	378	329	265	196
Control	461	458	454	450	445	436	412	375	332	302	277

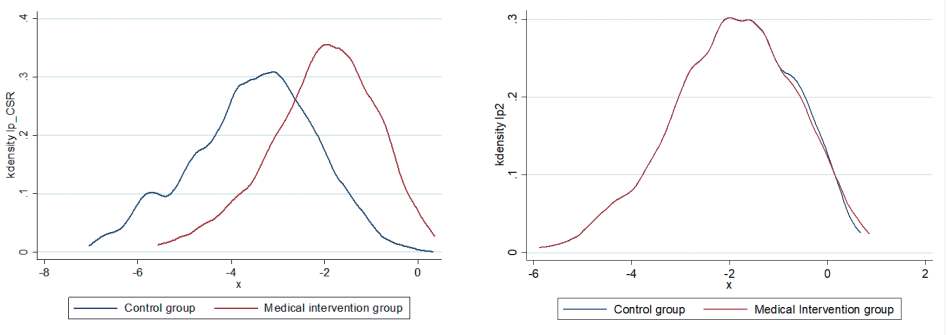
Figure 2B



	0	1	2	3	4	5	6	7	8	9	10
Revasc	129	124	117	114	113	110	105	103	94	68	52
Control	129	128	124	124	123	120	114	107	96	87	79

Figure 3A

A **B**

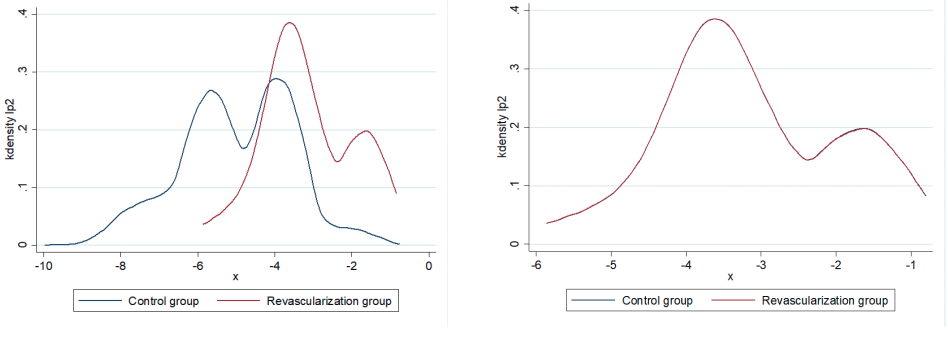


A. Medical intervention group versus Rotterdam Study control group before the Greedy propensity score matching analysis

B. Medical intervention group versus Rotterdam Study control group after the Greedy propensity score matching analysis

Figure 3B

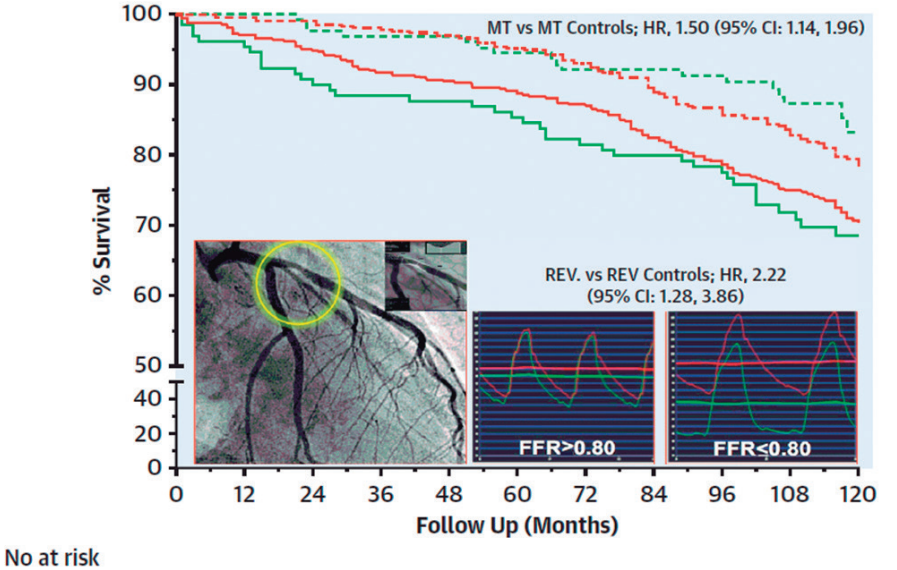
A **B**



A. Revascularization intervention group versus Rotterdam Study control group before the Greedy propensity score matching analysis

B. Revascularization intervention group versus Rotterdam Study control group after the Greedy propensity score matching analysis

Central Illustration



No at risk

MT	461	447	437	421	415	409	400	378	329	265	196
MT Controls	461	458	454	450	445	436	412	375	332	302	277
REV	129	124	117	114	113	110	105	103	94	68	52
REV Controls	129	128	124	124	123	120	114	107	96	87	79

Log-rank test MT vs REV $p > 0.05$; log-rank test both MT and REV vs their control groups $p < 0.01$

MT — MT Controls — REV — REV Controls

TABLES

Table 1: Baseline characteristics of the MT and REV groups of patients

	MT group n=564	REV group n=165
Age (year)	69 ± 10.9	67 ± 10.1
Male [a] (%)	55	76
BMI, (kg/m^2)	27 ± 4	27 ± 4
Diabetes (%)	17%	19%
Smoking status (%) [a]	35%	46%
Dyslipidemia (%)	57.6%	58.4%
Hypertension (%)	50.5%	48.8%
Family History of CAD (%)	5.7%	8.4%
Follow-up time, years, mean (SD)	8.30±2.53	8.05±2.85

		MT group n=564	REV group n=165
LVEF (SD)		71±13	69% ± 15
	<30%	2.5%	1.9%
	30-50%	8.5%	7.1%
	>50%	89.0%	91.0%
Diameter stenosis		39.8%±10.4%	54.0%±12.1%
Range of Diameter Stenosis[a]			
	30%–50%	77.3%	23.6%
	50%–70%	22.6%	76.4%
FFR [a]		0.87±0.05	0.71±0.08

Values are mean± SD or %. BMI= body mass index; CAD = coronary artery disease; FFR= fractional flow reserve; LVEF=left ventricular ejection fraction.
[a] = P value<0.05 between MT group and REV group.

Table 2: Clinical end-points at 10 years in the MT and the REV groups.

End Points	MT Group (n=564)	REV Group (n=165)	Hazard Ratio (95%CI)
Primary Composite end point	154 (27%)	74 (44%)	0.56 (0.37-0.71) p<0.0001
All Cause Death	132(23%)	39(23%)	0.96(0.67-1.38) p=0.85
Mocardial Infarction	7(1.2%)	12(7%)	0.17(0.04-0.31) p<0.0001
Target vessel MI	4(0.7 %)	5(3%)	
Death /MI	106(18.7%)	49(29%)	0.65(0.43-0.91) p=0.014
Target vessel revascularization	53(9%)	38(23%)	0.37 (0.17-0.47) p<0.0001
PCI			
All	33(5.3%)	30(18%)	
CABG			
All	20(3.5%)	8(4.8%)	

CHAPTER 3B

Mendelian randomization of markers of cardiac structure and function

CHAPTER 3B.1

Mendelian randomization of DHEAs and NT-Pro-BNP

Abstract submitted European Society Cardiology Congress 2020
Submitted to Circulation Research.

ABSTRACT

BACKGROUND: Observational evidence indicates an inverse association between the levels of the most abundant hormone in the human body, dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAs) and N-terminal pro B-type natriuretic peptide (NT-ProBNP). We aimed to generate estimates of the associations of DHEA and DHEAs (exposures) with NT-proBNP (outcome) that were free from confounding and reverse causation, and thus to assess the potential causal role of this endogenous sex hormone.

METHODS: Serum DHEA, DHEAs and NT-proBNP were assessed in 7,390 men and women free of cardiovascular diseases from the prospective population-based Rotterdam Study. DHEA, DHEAS and NT-proBNP were naturally log transformed. Regression coefficients and 95% confidence intervals (CI) were calculated from multivariable linear regression models adjusting for confounders to explore the cross-sectional association of DHEA and DHEAs with NT-proBNP. To investigate the causal association between DHEAs and NT-proBNP, allele score of exposure was used as an instrumental variable to perform a Mendelian Randomization (MR) analysis using two-stage least squares (2SLS) method. Pleiotropic effect was evaluated through Egger plots. A pathway analysis was performed to search for common biological paths linking these two hormones.

RESULTS: In models adjusted for multiple confounders (age, sex, lifestyle and cardiovascular risk factors), high levels of DHEA (β=-0.146, 95%CI: -0.190; -0.101, p<0.001) or DHEAs (β=-0.214, 95%CI: -0.262; -0.166, p<0.001) were associated with lower levels of NT-proBNP. Genetic risk score of DHEAs explained 0.75% and 29.39% variance of the circulating levels of NT-proBNP in crude and full adjusted models, respectively. The Mendelian Randomization analysis showed evidence for a causal association between DHEAs and NT-proBNP, with a causal coefficient of -0.450 (95% CI: -0.792; -0.107, p<0.010). Sex differences were observed with significant association only in women. Pathway analysis indicated inflammatory and immunologic pathways as common networks linking genes associated with DHEAs and NT-pro-BNP.

CONCLUSIONS: The causal association between DHEAs and NT-proBNP observed in this study suggests new metabolic pathways linking DHEAs with NT-proBNP. Our results should stimulate future research to evaluate the potential role of DHEAs in prevention and management of chronic heart failure.

Key words: Dehydroepiandrosterone; Natriuretic Peptides; Hormones; Biomarkers; Mendelian Randomization.

INTRODUCTION

DHEA and DHEAs are the most abundant sex hormones with serum concentrations up to 20-fold higher than the other sex steroids[1]. Plasma levels of DHEAs increase after birth reaching the peak by the second decade of life, afterwards, serum levels of DHEAs have a stable decline and, by the age of 80 years old, concentration drops to 10-20% of peak levels[1]. Emerging evidence indicates an association between low DHEAs, impaired longevity and common age-related diseases, including cardiovascular disease (CVD)[2]. Pooled estimates from several studies showed low DHEAs to be associated with a 47% higher risk of future CVD mortality events[2]. Furthermore, plasma levels of DHEAs are also decreased in proportion to the severity of heart failure (HF), which is the final common pathway of the majority of CVD[3]. B-type natriuretic peptide (BNP) and its hormonally inactive N-terminal portion (NT-proBNP) are sensitive biochemical markers of HF, particularly of left ventricular dysfunction and have similar diagnostic and prognostic accuracy in CVDs[4]. BNP is released from the myocardium in response to increased mechanical stress in order to maintain cardiac function by mediating vasodilation, natriuresis, and via its anti-fibrotic effects[5].

Emerging evidence showed that endogenous sex hormones levels play a role in the regulation of natriuretic peptides (NP); oestrogens may exert a stimulating effect on the NP system, while androgens may exert an inhibitory effect on the NP system[6]. In line with previous evidence from observational studies, we have showed in the Rotterdam Study inverse associations of DHEA and DHEAs with serum NT-proBNP levels in postmenopausal women without CVD[7]. Similarly plasma level of DHEAs was significantly inversely correlated with plasma levels of BNP independently of age and other clinical variables in subjects with HF[1]. In line with this, the experimental evidence from human heart showed that cardiac production of DHEA was suppressed in the failing heart[3]. Evidence from animals showed that DHEA significantly inhibited BNP mRNA levels in a neonatal rat cardiocyte culture system[3].

However, due to observational nature of previous studies affected by the possibility of residual confounding and reverse causation, it is not possible to draw conclusion regarding the causal association between DHEAs and NPs. Mendelian randomization (MR) method may be used to study the causal associations in presence of such limitations. The method is considered as a 'natural' randomized control trial since it uses selected common genetic variants related to a specific exposure of interest as an instrumental variable to evaluate causality between exposure and outcome. Since genotypes are assorted randomly during meiosis, MR avoids the issue of reverse causality. In addition, the distribution of genetic variants is thought to be unrelated to confounders, a common source of false positives in epidemiological studies[8]. Although, the physiological function of DHEAs and its importance in maintaining health are poorly understood, several common single nucleotide polymorphisms (SNPs) were associated with changes in gene expression levels, and the related genes are connected to biological pathways linking DHEAs with ageing[9,10].

Therefore, we aimed to study the causal association between serum DHEA(s) and NT-proBNP, in subjects free of cardiovascular diseases, using the MR approach of identified genetic variants combined into genetic risk score (GRS) as an instrumental variable.

METHODS

Study Population

This study was conducted among participants of the prospective population-based Rotterdam Study (RS)[11]. RS is a study of individuals aged 45 and over living in the Ommoord district of Rotterdam, the Netherlands. The rationale and design of RS have been described previously. In brief, all residents of the Ommoord district aged 55 or older were invited to participate (n=10,215). At baseline (1990-1993), 7,983 participants were included (RS-I). In 2000, an additional 3,011 participants were enrolled (RS-II), consisting of persons living in the study district who had become 55 years of age. A second extension of the cohort was initiated in 2006, in which 3,932 participants aged 45 or older were included (RS-III). Follow-up visits were held every 3-5 years, with follow-up for a variety of diseases. The RS has been approved by the medical ethics committee according to the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants[11].

Population for Analyses

The present study includes data from individuals from the third visit of the first cohort of the RS (RS-I-3), and from the first visits of the second (RS-II-1) and the third cohort (RS-III-1). There were 11,732 subjects eligible for the analysis. Of those 4,342 participants were excluded because (i) information was not available on NT-proBNP (n=814), DHEAs (n=205) or on the genetic risk score (n=2,318); (ii) they had prevalent cardiovascular disease (coronary heart disease, stroke or HF) (n=947); and (iv) there was no information on history of cardiovascular disease (n=58). Finally, there were 7,390 participants left for the analysis (**Figure 1**).

Exposure and Outcome Measurement

DHEA and DHEAs were exposure variables. They were measured on a Waters XEVO-TQ-S system (Waters, Milford, MA, USA) using CHS™ MSMS Steroids Kit (Perkin Elmer, Turku, Finland). Inter-assay coefficients of variation of androstenedione, DHEAs and DHEA were <6.5%. NT-proBNP levels were obtained from serum. After blood collection, samples were left to clot for 30 minutes and then centrifuged for 20 minutes at 3000 rotations per minute at 4ºC. Serum was stored at -80°C. NT-proBNP was measured using a commercially available electrochemiluminescence immunoassay (Elecsys proBNP, F. Hoffman-La Roche Ltd., Basel, Switzerland) on an Elecsys 2010 analyser. Precision, analytical sensitivity and stability characteristics of the system have been previously described[12].

Assessment of Covariates

At baseline interview, all participants provided information on current health status, medical history, medication use, alcohol intake, smoking and physical activity. History of cardiovascular disease was defined as the history of coronary heart disease (myocardial infarction, revascularization, coronary artery bypass graft surgery or percutaneous coronary intervention) and was verified from the medical records of the general practitioner. Diabetes mellitus was defined as the use of blood glucose–lowering medications or a random non-fasting glucose >11.1 mmol/L[13]. Antihypertensive or antidiabetic therapy and statins were collected by questionnaire during home interview. Alcohol intake was assessed in grams of ethanol per day and grouped into 4 categories (0-0.99, 1-19.9, 20-39.9 and ≥40 g/day); smoking status was assessed by asking participants whether they were

current smokers of cigarettes, cigars, or pipe and were classified (yes/no). Physical activity was assessed with adapted version of the Zutphen Physical Activity Questionnaire[14]. Every activity mentioned in the questionnaire was attributed a MET-value according to the 2011 Compendium described in detail elsewhere[15]. Blood pressure was measured in sitting position on the right upper arm with a random-zero sphygmomanometer. Body mass index (BMI) was calculated as weight (kg) divided by height square (m^2). Glomerular filtration rate (eGFR) was estimated using the simplified Modification of Diet in Renal Disease (MDRD) equation[16]. Thyroid stimulating hormone (TSH) was measured on the Vitros Eci (Ortho Diagnostics). Insulin, glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triacylglycerol (TG) and C-reactive protein (CRP) were measured on the COBAS 8000 Modular Analyzer (Roche Diagnostics GmbH). Corresponding interassay coefficients of variations are as follows: TSH<13.2%, insulin <8%, glucose <1.4%, lipids <2.1% and CRP <16.9%. LDL-cholesterol level was estimated indirectly from measurements of total cholesterol, HDL and triglycerides by means of the Friedewald equation[17]. Total estradiol (TE) levels were measured with a radioimmunoassay and sex hormone binding globulin (SHBG) by means of the Immulite platform (Diagnostics Products Corporation Breda, the Netherlands). Minimum detection limit for estradiol was 18.35pmol/l. Undetectable estradiol was scored as 18.35pmol/l. Serum levels of total testosterone (TT) were measured with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Corresponding interassay coefficients of variations for TE, SHBG and TT are <7%, <5%, and <5%. Free androgen index (FAI), calculated as (T/SHBG)*100 is used as a surrogate measure of bioavailable testosterone (BT). All biochemical parameters were assessed in fasting serum.

Genotyping

Genotyping was conducted in all three cohorts using the Illumina Infinium HumanHap550K Beadchip in RS-I and RS-II and the Illumina Infinitum HumanHap 610 Quad chip in RS-III at the Genetic Laboratory of the Erasmus MC, Department of Internal Medicine, Rotterdam, The Netherlands. Participants were excluded if they had excess autosomal heterozygosity, mismatch between called and phenotypic sex, or recognized as being outlier with identical-by-state clustering analysis. Moreover, SNPs with allele frequency ≤1%, Hardy–Weinberg equilibrium $p<10^{-5}$, or SNP call rate ≤90% were excluded. Imputation was done with reference to HapMap release 22 CEU (Utah residents of northern and western European ancestry) using the maximum likelihood method implemented in Markov Chain based haplotyper (version 1.0.15).

Construction of DHEAs Genetic Risk Score (GRS)

We searched PubMed using key words 'genome-wide association study', 'GWAS', 'DHEAs', 'Dehydroepiandrosterone sulfate', GWAS catalog, and Genome- Wide Repository of Associations between SNPs and Phenotypes (GRASP). We identified two large genome-wide association studies conducted on >14,846 individuals of European descent[9,10]. Nine SNPs identified from these GWAS were used to build the genetic risk score of DHEAs (rs148982377, rs11761528, rs2637125, rs7181230, rs2497306, rs2185570, rs740160, rs17277546 and rs6738028) (Supplemental Table 1). The effect allele (coded 0–2) was the DHEAs raising allele. A weighted GRS was calculated by multiplying the number of risk alleles at each locus by the corresponding reported β coefficient from the previous GWAS and then summing the products[18]. The total score was then divided by the average effect size multiplied by 100 to rescale the scores to a range between 0 and 100. We could not identify genome-wide association studies published for DHEA and, therefore, we could only build a genetic risk score for DHEAs.

Pathway analysis

To explore the pathways in which the genes associated with DHEAs and NT-proBNP may be related, we used Ingenuity Pathway Analysis (IPA) (http://www.ingenuity.com/products/ipa/). IPA is a web-based functional analysis tool to identify the biological mechanisms, pathways, and functions most relevant to the genes of interest. To this end, we uploaded the list of DHEAs-associated genes and performed a core analysis with the default settings in IPA. We mapped these genes to biological functions or diseases. We further sought to determine whether these genes are enriched in specific networks linking NT-proBNP and DHEAs to heart failure (or cardiovascular disease). The p-values in IPA are calculated using the right-tailed Fisher Exact Test and a p-value less than 0.05 indicates a statistically significant, non-random association.

Statistical Analyses

Cross-sectional Analyses

DHEA, DHEAs, NT-proBNP, hsCRP, 17-hydroxyprogesterone and cortisol levels were log-transformed using a natural log to obtain normal distribution. Cross-sectional association between log transformed continuous DHEA/DHEAs and

NT proBNP was assessed using ordinal linear regression (OLR) models. Betas were calculated after adjusting for age, sex, interaction term between sex and DHEA/DHEAs (sex*DHEAs p=0.000 and sex*DHEA p=0.002), RS cohort, BMI, physical activity, smoking, alcohol, cholesterol, statin use, glucose, systolic blood pressure, antihypertensive therapy, type 2 diabetes (T2D), eGFR, hsCRP, 17-hydroxyprogesterone and cortisol. Additionally, to explore potential sex differences we run the analysis stratified by gender.

Association of DHEAs Genetic Risk score and NT-proBNP

The MR approach is used to investigate the causality of associations between DHEAs and NT-proBNP. Since no SNPs genome-wide significant for DHEA have been published, we could not assess the causality between DHEA and NT-proBNP. In the current study we used the genetic risk score (GRS) of DHEAs (calculated based on nine publically available SNPs) as an instrumental variable (IV). Valid instrumental variable is a factor that is associated with the exposure, but is not associated with any confounder of the exposure–outcome association, nor is there any pathway by which the IV can influence the outcome other than via the exposure of interest/no pleiotropy[19] (Figure 2). Given a continuous outcome (NT-proBNP) and assuming the linear associations between DHEAs and NT-proBNP without interaction, we estimated the casual association between GRS of DHEAs and serum NT-proBNP through a 2-stage least squares (2SLS) regression[20]. The 2SLS estimation proceeds by first fitting the regression of DHEAs (exposure) on the GRS of DHEAs (instrument), and the second step assesses the association of DHEAs with NT-proBNP (outcome) on the fitted values from the first-stage regression. Within these models, age, sex, RS cohort, BMI, physical activity, smoking, alcohol, total cholesterol, statin use, glucose, systolic blood pressure, antihypertensive therapy, T2D, glomerular filtration rate (eGFR), hsCRP, 17-hydroxyprogesterone and cortisol were included as covariates in order to generate estimates from the IV analyses that were comparable to those from the observational regressions. We also evaluated the instrument strength using F-statistics from the first-stage regressions, where F-statistics >10 has been used to indicate sufficient strength, and by R^2 (%) as a measure of the percentage contribution of GRS to the variation of NT-proBNP levels. Standard MR analysis assume that genetic instruments only influence the outcome (i.e. NT-proBNP) through the exposure of interest (serum DHEAs), however, DHEAs associated SNPs may influence serum NT-proBNP through pathways other than serum DHEAs concentration. We therefore tested the robustness of our findings by MR Egger regression which helps to control for biases though horizontal pleiotropy. The slope of the weighted regression

line provides an estimate of the causal effect of the exposure on the outcome free from the effects of horizontal pleiotropy. While the intercept in the regression is a function of extent of directional pleiotropy in the data aggregated across all the different variants used in the analysis, and statistical tests of the degree to which the intercept differs from zero are using to test the overall presence of directional pleiotropy in the data[21]. In case of significant intercept (and therefore evidence of directional effects) the estimate from the Eggers would be a better estimate, however, if no evidence of directional pleiotropy then the 2SLS is better powered.

To validate the causal estimate derived from the 2SLS method, we obtained MR estimations using the Wald ratio method. As follows, two normal linear regressions were performed: regression of DHEAs on GRS of DHEAs (the first-stage regression) and regression of NT-proBNP on GRS of DHEAs (reduced-form regression). The ratio of these estimates (the Wald estimate) and corresponding confidence intervals were obtained using *suest* and *nlcom* commands in Stata[22], this estimations were adjusted by age, sex and RS cohort. A multiple imputation (chained equations method) was applied for missing data. For most baseline clinical variables, <2% was missing, whereas this was up to 12% and 26% for self-reported variables such as physical activity and alcohol intake, respectively. All statistical analyses were carried out using Stata/IC statistical Software, version 15 and MR package of R software.

RESULTS

Baseline characteristics of the population used for analysis are shown in **Table 1**. Median age (Q1-Q3) of participants was 63 (58-71) years, and 59.9% of included subjects were women. The median levels of NT-proBNP were 7.9 pmol/l (Q1=4.3; Q3=14.9), DHEA 9.6 nmol/l (Q1=6.2; Q3=15.0), DHEAs 2,099nmol/l (Q1=1,257; Q3=3,365) and DHEAs GRS 48.2 (Q1=45.1; Q3=50.8).

Observational associations between DHEA, DHEAs and NT-proBNP

Based on 7,390 subjects, we observed an inverse association between serum DHEAs levels and NT-proBNP levels. In crude model for each one-point increase in levels of natural log transformed DHEAs, NT-proBNP levels decreased -0.395 (β; 95%CI: -0.423; -0.366; p<0.001). In multivariable linear regression model (adjusted for age, sex, interactions of DHEAs*sex, RS cohort, BMI, physical activity, smoking, alcohol, cholesterol, statin use, glucose, systolic blood pressure, antihypertensive

therapy, diabetes mellitus type 2, eGFR, hsCRP, 17-hydroxyprogesterone and cortisol) for each one-point increase in levels of natural log transformed of DHEAs, NT-proBNP levels decreased -0.214 (β; 95%CI: -0.262; -0.166; p<0.001) (**Table 2**). Stratification by gender did not materially change the results. Among both, men and women in fully adjusted models, high levels of serum DHEAs were associated with low levels of NT-proBNP levels (Supplemental table 2). Furthermore, in fully adjusted model for each one-point increase in levels of natural log transformed DHEA, NT-proBNP levels decreased by -0.146 (β; 95%CI: -0.190; -0.101; p<0.001), also, gender stratification did not yield any changes (Supplemental **Table S3**). Assumptions of linearity, homoscedasticity and normality were assessed; but no major violations were observed.

Causal estimates for the effect of DHEAs genetic risk score on NT-proBNP levels

A weighted gene score composed of 9 genetic variants for elevating DHEAs was used as the genetic instrument. DHEAs GRS was strongly associated with circulating NT-proBNP levels, explaining on average 0.75% and 29.39% of NT-proBNP variation, in crude and adjusted models, respectively, with F-statistics=58.26 (**Table 2**), indicating that GRS is unlikely to be affected by weak instrument bias. Neither individual genetic variants nor the gene score were associated with potential confounders including sex, age and BMI (Supplemental **Table S4**). Also, using ordinal lineal regression model adjusted by age, sex and RS cohort, we investigated the association between individual DHEAs SNPs and NT-proBNP and none of the SNPs was statistically significant at p-value<0.05 (Supplemental **Table S5**). In the MR analysis, applying 2SLS approach in the entire study group, using DHEAs genetic risk score as the instrumental variable, significant causal association was observed between DHEAs and NT-proBNP levels, either in the crude or adjusted analysis. Genetically predisposed higher levels of DHEAs were associated with decreased serum NT-proBNP levels [crude model β=-0.530 (95%CI:-0.863; -0.198; p=0.002) and adjusted model β=-0.450 (95%CI:-0.792; -0.107; p=0.010)] (**Table 2**). As in the observational analysis, we found significant interaction between DHEAs and sex we run the 2SLS analysis separate for men and women. After stratification by sex, in both men and women, results were similar to overall findings (Supplemental **Table S2**). However, in men the value of F statistics the GRS of DHEAs was close to value considered as a weak instrument (F <10), which could be due to low power, as we confirmed in the power calculation analysis (Supplemental **Table S2**). We applied an extension of MR, Eggers regression to test for horizontal pleiotropy. The intercept of the

MR-Egger regression captures the average pleiotropic effect across all genetic variants. None of the analyses performed had a significant intercept indicating no directional pleiotropy (Supplemental **Table S6**).

Pathways analysis

The IPA core analysis indicated that the studied genes are directly or indirectly linked with inflammatory-related pathways (p-value=$3x10^{-7}$) and metabolic pathways (p-value=$7.8x10^{-3}$ to 6.7×10^{-5}) (supplementary **Table S7** and **Figure S3**). We further generated common networks linking the genes associated with NT-proBNP and DHEAs to heart failure and its related phenotypes. As shown in supplementary **Table S7**, these are both linked via NPPA and NPPB genes from natriuretic peptide to ARPC1A gene from DHEAs, which might indicating a potential mechanism explaining the association between NT-proBNP and DHEAs. Indeed, NPPA and NPPB are associated with cardiovascular morbidities as acute and chronic heart failure, atrial fibrillation and arrhythmia (Supplemental **Figure S3**). Further studies are need to investigate the exact mechanisms and pathways linking these two hormones.

DISCUSSION

Overall, in this large population-based study among individuals free of CVD we found statistically significant inverse associations between DHEA and DHEAs and serum NT-proBNP. In Mendelian randomization approach genetically predisposed higher levels of DHEAs were associated with lower NT-proBNP concentrations; therefore, providing evidence for potential causal, inverse association between DHEAs and NT-proBNP.

Our findings complement the preceding publication from the RS, where cross-sectional data from postmenopausal women free of CVD disease, have shown inverse association between DHEA and DHEAs and serum NT-proBNP[7]. Also, our results are in line with previous observational data. Several epidemiological studies have demonstrated an association between low serum levels of DHEAs with elevated CVD risk[23,24], cardiovascular morbidity[25-27], coronary artery disease[28.29] and vascular atherosclerotic disease[30]. Moriyama et al. reported positive association between DHEAs levels and left ventricular ejection fraction (LVEF), as well as inverse association with BNP levels in an Asian population, independently of age and other clinical variables[1]. Also, Kawano et al. showed DHEAs levels to increase

upon improvement of ventricular function in patients undergoing HF treatment[31]. It has also been reported that DHEAs can be produced in cardiomyocytes of structurally healthy hearts, but not in failing hearts[32].

Despite increasing evidence suggesting its beneficial cardiovascular effects, an intracellular steroid hormone receptor for DHEA has not been identified[3]. Recent reports suggested specific DHEA-binding sites in cardiovascular tissue[33,34], and that this putative receptor is present in the rat heart[34]. However, it is unclear if DHEA directly exerts its effects or if it acts after conversion to testosterone/17β-estradiol, via binding specific receptors for testosterone and 17β-estradiol that are present in the heart[35]. The inverse association between DHEAs and NT-proBNP can be explained by the opposite biological effect they produce. DHEA and DHEAs may play a beneficial role in cardiovascular system through modulation of several processes such as nitric oxide production stimulation, oxidation stress inhibition, prevention of vascular remodelling, stimulated vasodilation[36]. Conversely, increased NPs production at both auricular and ventricular level, and progressively according to ventricular dysfunction, has been previously evidenced in patients with HF, which is in turn associated with increased oxidative stress, that might alter the electron transport mechanism at P450C17 cytochrome level, selectively suppressing 17,20 lyase enzyme activity, resulting in decreased DHEAs serum levels[23].

Recently, nine common genetic variants were associated with serum DHEAs, suggesting its key role in aging mechanisms[37]. Genes at or near these genetic variants include ARPC1A, BCL2L11, ZKSCAN5, ZNF789, TRIM4, HHEX, CYP2C9, BMF and SULT2A1. These genes have various associations with steroid hormone metabolism co-morbidities of ageing including type 2 diabetes, lymphoma, actin filament assembly, drug and xenobiotic metabolism, and zinc finger proteins—suggesting a wider functional role for DHEAs than previously thought[6]. Using DHEAs genetic risk score as an IV, our findings suggest that genetically predisposed higher DHEAs concentrations are inversely associated with NT-proBNP levels. Therefore, there might be a causal association between DHEAs and NPs. Still, the common biochemical pathways that link the metabolism of these two hormones are largely unknown, and should further be investigated. Our pathways analysis suggested common biological paths that might link DHEAs and NT-pro-BNP, in particular via NPPA and NPPB from natriuretic peptide with ARC1A from DHEAs, with multiple cardiovascular morbidities.

Strengths and limitations

To the best of our knowledge, this is the first study to examine the causal association between DHEAs and NT-proBNP levels in a large population based sample of CVD free men and women. Also, DHEA and DHEAs are measured using chromatography-tandem mass spectrometry, which is at the moment considered to be a gold standard method[38]. Although MR is considered as a flexible and robust statistical method, there is a number of MR limitations which need to be considered, also, the limitations of the observational part of our analysis merits further discussion. First, in the RS, serum BNP levels were not measured, but solely its inactive precursor NT-proBNP. However, recent systematic reviews and meta-analyses demonstrated that both BNP and NT-proBNP have similar diagnostic and prognostic accuracy in CVDs[4]. Second, there were no publically available SNPs on DHEA, therefore, we were not able to calculate GRS of DHEA and we could not study the causal association between DHEA and NT-proBNP. Also, within the RS we did not identify any SNPS associated with serum DHEA. However, DHEAs is more stable sulfate ester of DHEA, and it can be converted back to DHEA by steroid sulfatase, which can be considered a good proxy of the association between DHEA and NT-proBNP as confirmed in our regression analysis (cross-sectional associations between DHEA and DHEAs and NT-proBNP were in line)[39]. Third, calculation of allele score is considered to be a good approach to avoid weak IV bias for reasons and also may increase the power and simplicity[18]. However, due to complex biology, the effects of all the variants in an allele score may not be well known, the instrumental variable assumptions may not be satisfied for all the variants[18]. Weakly associated instruments (F statistics < 10) can bias causal estimates towards the observational estimate for one-sample MR. Indeed, the strength of the GRS as an instrument, measured by the F statistic was satisfactory overall, and in females, but in men F statistics was close to 10 indicating that in males, DHEAS GRS might be a weak instrument. However, we consider this could be due to that the association of androgens with NT-pro-BNP levels are more robust in women than men, as has been previously reported, and low power, as we confirmed in the power calculation analysis. Fourth, an important assumption of Mendelian randomization is that the genetic variant must mediate its effect on outcome only via the risk factor, i.e., the genetic variant shows no pleiotropic effects. Therefore, this assumption cannot be proven formally in practice because of incomplete knowledge of the underlying biology. However, we applied an extension of MR approach: MR Egger regression, to test for the causal effect free of pleiotropy. In simple words, provided the underlying assumptions are met, the slope of the MR Egger regression analysis should yield an estimate of the causal effect of DHEA on NT-proBNP that is free from any confounding

effects due to horizontal pleiotropy. However, it is important to mention that the validity of MR Egger regression rests on the 'INSIDE assumption' (INstrument Strength is Independent of Direct Effect) which states that across all instruments there should be no correlation between the strength with which the instrument proxies the exposure of interest, and its degree of association with the outcome via pathways other than through the exposure[40]. This is a weaker requirement than the exclusion restriction criterion in normal MR which postulates that SNPs may only affect the outcome (NT-proBNP) through the exposure of interest (serum DHEAs), and so MR Egger regression is likely to be more robust to horizontal pleiotropy than standard MR approaches, although this appears to come at the cost of decreased power to detect a causal effect in one sample MR[40]. However, the MR-Egger intercept indicated no presence of horizontal pleiotropy.

Clinical implications

In cross-sectional analysis DHEA and DHEAs were significantly inversely associated with serum NT-ProBNP levels. Causal association we have observed between DHEAs and NT-proBNP suggests a new metabolic pathway linking DHEAs with NT-proBNP, which merits detailed experimental investigation in the future. Altering the serum DHEAs might play an important role in prevention and management of chronic heart failure, therefore, after exploring the biology behind our findings; clinical studies shall address health benefit of modifying serum DHEAs in subjects with heart failure.

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Figure 1. Flowchart for selection of study participants (n=7,390)

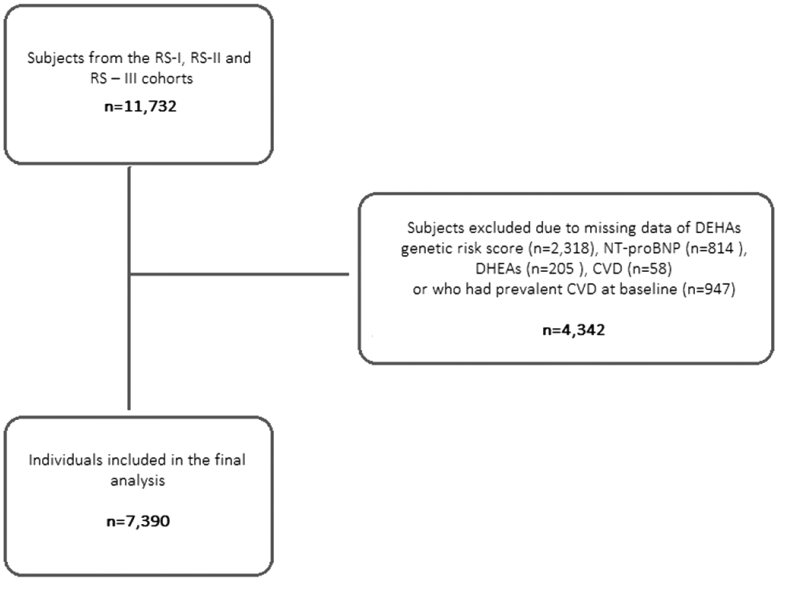
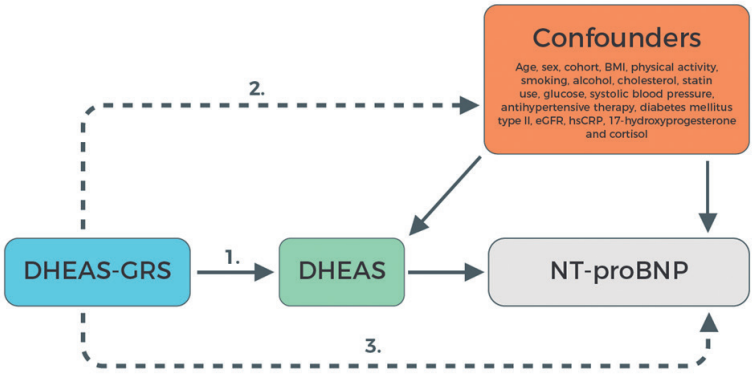


Figure 2. Assessing the causality of DHEAS and NT-proBNP levels



GRS of DHEAs is associated with DHEAs (exposure)
GRS of DHEAs is not associated with measured or unmeasured confounders
GRS of DHEAs is only associated with NT-proBNP (outcome) through the exposure

Table 1. Characteristics of study population.

Characteristics	Median (Q1-Q3)/ n (%)	Characteristics	Median (Q1-Q3)/ n (%)
Age (years)	63 (58-71)	**Health indicators**	
Sex (female)	4,431 (59.96)		
Health behaviours			
Body mass index (kg/m^2)	27 (24-29)	Systolic blood pressure (mmHg)	138 (125-152)
Smoking (yes)	1,528 (20.68)	Diastolic blood pressure (mmHg)	79 (71-86)
Alcohol intake (g/day)		Total cholesterol (mmol/l)	5.8 (5.1-6.4)
0-0.99	2,541 (34.38)	HDL-C (mmol/l)	1.4 (1.1-1.7)
1-19.9	2,559 (34.63)	Triglycerides in serum (mmol/l)	1.3 (1.0-1.8)
20-39.9	1,890 (25.58)	Fasting blood glucose (mg/dl)	5.5 (5.1-6.0)
≥40	400 (5.41)	Insuline (pmol/l)	71 (50-103)
Physical activity (total MET hours)	70 (41-103)	hs-CRP (mg/ml)	1.6 (0.6-3.5)

Characteristics	Median (Q1-Q3)/ n (%)	Characteristics	Median (Q1-Q3)/ n (%)
Hormones		eGFR (mL/min/1.73m^2)	80 (69-90)
Estradiol (pmol/l)	63 (25-103)	Antihypertensive use (yes)	2,085 (28.21)
Testosterone (nmol/l)	1.3 (0.7-15.1)	Serum lipid lowering medication (yes)	980 (13.26)
SHBG (nmol/l)	52 (38-73)		
DHEA (nmol/l)	9.6 (6.2-15.0)	Prevalent diabetes mellitus	781 (10.57)
DHEAs (nmol/l)	2,099 (1,257-3,365)		
DHEAs GRS	48.2 (45.1-50.8)		
Androstenedione	2.7 (2.0-3.6)		
17-hydroxyprogesterone (nmol/l)	1.5 (0.8-2.7)		
NT-proBNP	7.9 (4.3-14.9)		

Values are presented absolute value and percentage for categorical variables, and median (25th-75th quartile) for continuous variables.
Abbreviations: HDL=high density lipoprotein colesterol; hs-CRP=high-sensitivity C reactive protein; eGFR=glomerular filtration rate; SHBG= sex hormone binding globulin; DHEA=dehydroepiandrosterone; DHEAs=dehydroepiandrosterone sulfate; DHEAs GRS= dehydroepiandrosterone sulfate genetic risk score; TSH=thyroid stimulating hormone; NT-proBNP=amino-terminal pro-B-type natriuretic peptide.

Table 2. Summary statistics describing observational and causal relationship DHEAs and NT-proBNP (n=7,390)

Method	β	SE Error	95% CI	p-value	F-statistics	R^2
Overall Crude Model						
OLR	-0.395	0.014	-0.423; -0.366	0.000	----	0.0898
2SLS	-0.530	0.169	-0.863; -0.198	0.002	56.02	0.0075
Overall Adjusted Model						
OLR	-0.214	0.024	-0.262; -0.166	0.000	----	0.3252
2SLS	-0.450	0.174	-0.792; -0.107	0.010	58.28	0.2939

Coefficients represent the decrease in log-NT-proBNP for each unit increase in log-DHEAs.

Adjusted model: age, sex, sex*DHEAs, cohort, BMI, physical activity, smoking, alcohol, total cholesterol, statin use, glucose, systolic blood pressure, antihypertensive therapy, diabetes mellitus type 2, glomerular filtration rate (eGFR), hsCRP, 17-hydroxyprogesterone and cortisol; DHEAs, NT-proBNP, hsCRP, 17-hydroxyprogesterone and cortisol levels were log-transformed using a natural-log.

Abbreviations: OLR= Ordinal lineal regression (Observational analysis); 2SLS=Two-stage least squares regression; DHEAs=dehydroepiandrosterone sulfate; NT-proBNP=amino-terminal pro-B-type natriuretic peptide; BMI=body mass index; eGFR: glomerular filtration rate; hs-CRP=high-sensitivity C reactive protein.

Supplemental Material

Supplemental Table 1. Genetic variants and alleles used in the genetic risk score of DHEAs

SNP	Gene	Coded	Beta
rs148982377	ZNF789	C	0.255
rs11761528	ZKSCAN5	T	-0.16
rs2637125	SULT2A1	A	-0.09
rs7181230	BMF	G	0.05
rs2497306	HHEX	C	-0.04
rs2185570	CYP2C9	C	-0.06
rs740160	ARPC1A	T	0.15
rs17277546	TRIM4;CYP3A43	A	-0.11
rs6738028	BCL2L11	G	-0.04

Supplemental table 2. Summary statistics describing observational and causal relationship DHEAs and NT-proBNP stratified by gender (n=7,390)

Method	β	SE Error	95% CI	p-value	F-statistics	R^2
Women Crude Model						
OLR	-0.268	0.017	-0.302; -0.234	0.000	----	0.0515
2SLS	-0.424	0.178	-0.773; -0.075	0.017	43.05	0.0096
Women Adjusted Model						
OLR	-0.088	0.016	-0.121; -0.055	0.000	----	0.2722
2SLS	-0.388	0.165	-0.714; -0.063	0.019	48.91	0.1911
Men Crude Model						
OLR	-0.494	0.029	-0.551; -0.436	0.000	----	0.0871
2SLS	-0.729	0.489	-1.688; 0.229	0.136	10.96	0.0037
Men Adjusted Model						
OLR	-0.145	0.029	-0.202; -0.088	0.000	----	0.3382
2SLS	-0.643	0.467	-1.560; 0.273	0.169	12.48	0.2588

Coefficients represent the decrease in log-NT-proBNP for each unit increase in log-DHEAs.
Adjusted model: age, cohort, BMI, physical activity, smoking, alcohol, total cholesterol, statin use, glucose, systolic blood pressure, antihypertensive therapy, diabetes mellitus type 2, glomerular filtration rate (eGFR), hsCRP, 17-hydroxyprogesterone and cortisol.
DHEAs, NT-proBNP, hsCRP, 17-hydroxyprogesterone and cortisol levels were log-transformed using a natural-log.
Abbreviations: OLR=Ordinal lineal regression (Observational analysis); 2SLS=Two-stage least squares regression; DHEAs=dehydroepiandrosterone sulfate; NT-proBNP=amino-terminal pro-B-type natriuretic peptide; BMI=body mass index; eGFR=glomerular filtration rate; hs-CRP=high-sensitivity C reactive protein.

Supplemental table 3. Associations between DHEA with the level of serum NT-proBNP in people free of CVD, the Rotterdam Study (n=7,357)

	NT-proBNP				
	β	95% CI	SE	p-value	R^2
All	-0.146	-0.190; -0.101	0.022	0.000	0.3216
Women	-0.097	-0.137; -0.057	0.020	0.000	0.2712
Men	-0.086	-0.150; -0.023	0.032	0.008	0.3343

Models adjusted by age, sex, sex*DHEA, cohort, BMI, physical activity, smoking, alcohol, total cholesterol, statin use, glucose, systolic blood pressure, antihypertensive therapy, diabetes mellitus type 2, glomerular filtration rate (eGFR), hsCRP, 17-hydroxy-progesterone and cortisol. Models of women and men the same adjustment except sex and sex*DHEA interaction. DHEA, NT-proBNP, hsCRP, 17-hydroxyprogesterone and cortisol levels were log-transformed using a natural-log.
Abbreviations: DHEA=Dehydroepiandrosterone; NT-proBNP= Amino-terminal pro-B-type natriuretic peptide; BMI=Body mass index; eGFR=glomerular filtration rate; hs-CRP=high-sensitivity C reactive protein.

Supplemental Table 4. Analysis of association of DHEAs genetic risk score with tested confounders by linear regression

DHEAs Genetic Risk score	Crude		Adjusted	
	β	p-value	β	p-value
Age	-0.009	0.072	-0.002	0.759
Sex	-0.190	0.059	-0.177	0.080
Cohort	0.126	0.026	0.107	0.165
Body mass index	0.010	0.375	0.009	0.419
Physical activity	-0.001	0.149	-0.0008	0.380
Smoking	0.116	0.340	0.080	0.518
Alcohol	0.014	0.794	-0.013	0.810
Total cholesterol	-0.018	0.710	0.007	0.885
Statin Use	-0.166	0.253	-0.200	0.175
Glucose	-0.006	0.855	-0.005	0.879
Systolic blood pressure	-0.0007	0.739	0.00005	0.981
Antihypertensive therapy	0.149	0.173	0.201	0.073
Diabetes mellitus, type 2	0.017	0.915	0.022	0.891
Glomerular filtration rate	0.001	0.581	-0.003	0.361
High-sensitivity C reactive protein	-0.012	0.777	0.020	0.642
17-hydroxyprogesterone	-0.013	0.835	-0.176	0.044
Cortisol	-0.092	0.521	-0.068	0.633

Adjusted by age, sex, cohort. *Statistically significant p-value=0.0055

Supplemental Table 5. Association of individual DHEAs SNPs with NT-proBNP levels in the Rotterdam Study.

NT-proBNP	β	p-value*
rs148982377	-0.013	0.744
rs11761528	-0.044	0.082
rs2637125	-0.028	0.143
rs7181230	0.018	0.217
rs2497306	-0.006	0.627
rs2185570	-0.013	0.520
rs740160	-0.050	0.150
rs17277546	0.001	0.953
rs6738028	-0.006	0.685

Adjusted by age, sex, cohort. *Statistically significant p-value=0.0055

Supplemental table 6. MR-Egger intercept

Method	β	SE Error	95% CI	p-value
Overall Crude Model				
MR-Egger (intercept α)	-0.016	0.013	-0.041; 0.008	0.195
Overall Adjusted Model				
MR-Egger (intercept α)	-0.006	0.010	-0.025; 0.013	0.543
Women Crude Model				
MR-Egger (intercept α)	-0.016	0.020	-0.054; 0.022	0.409
Women Adjusted Model				
MR-Egger (intercept α)	0.002	0.017	-0.031; 0.035	0.900
Men Crude Model				
MR-Egger (intercept α)	-0.015	0.018	-0.051; 0.021	0.410
Men Adjusted Model				
MR-Egger (intercept α)	0.000	0.015	-0.029; 0.029	0.982

Models adjusted by age, sex, cohort, BMI, physical activity, smoking, alcohol, total cholesterol, statin use, glucose, systolic blood pressure, antihypertensive therapy, diabetes mellitus type 2, glomerular filtration rate (eGFR), hsCRP, 17-hydroxyprogesterone and cortisol. DHEA, NT-proBNP, hsCRP, 17-hydroxyprogesterone and cortisol levels were log-transformed using a natural-log.

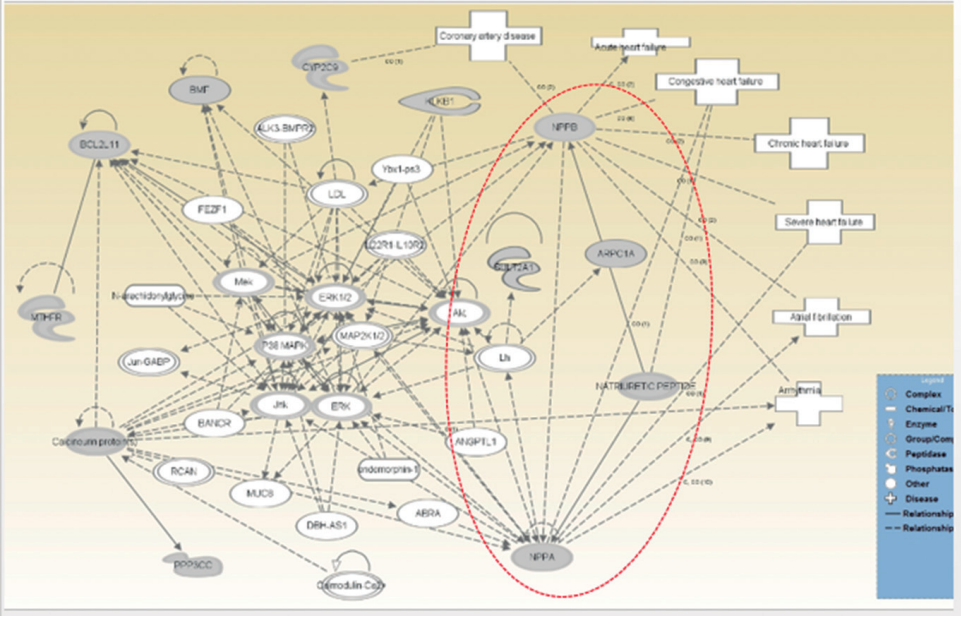
Abbreviations: DHEA=Dehydroepiandrosterone; NT-proBNP= Amino-terminal pro-B-type natriuretic peptide; BMI=Body mass index; eGFR=glomerular filtration rate; hs-CRP=high-sensitivity C reactive protein.

Supplemental table 7. IPA pathway analysis for genes associated with NT-proBNP and DHEAs

NT-proBNP		
Top Canonical Pathways	**p-value**	**Molecules**
Semaphorin Signaling in Neurons	3.82E-04	CFL2, RHOQ
ILK Signaling	5.12E-03	CFL2, RHOQ
AMPK Signaling	6.12 E-03	RAB1A, RAB2A
Top Upstream Regulators	**p-value**	
Inflammation	2.99E-07	
Top Diseases and Bio Function (Physiological System development and function)	**p-value**	**Molecules**
Nervous System Development and Function	4.98E-02, 5.54E-04	TGFBR1, CFL2, ALCAM , RHOQ SEMA3D
DEHAs		
Top Canonical Pathways	**p-value**	
Super pathway of Melatonin degradation	4.56E-04	
LPS/IL-1 Mediated inhibition of RXR Function	4.46E-03	
Xenobiotic Metabolism Signaling	7.80E-03	
Top Upstream Regulators	**p-value**	
Oxidation (NOX)	6.72E-05	
Top Diseases and Bio Function	**p-value**	
Organismal injury and abnormalities	4.95E-02	

The table shows the top canonical pathways for the genes associated with NT-proBNP and DEHAs using IPA. The p-values are calculated using the right-tailed Fisher Exact Test, a p-value < 0.05 indicates a statistically significant.

Supplemental Figure 3. Biological pathways linking DHEAs with Natriuretic peptide



Red Circle: Associated genes among DHEAs and natriuretic peptide to main cardiovascular morbidities.

CHAPTER 4

Epidemiological studies of aortic structural and function

CHAPTER 4.1

Sex-specific distributions and determinants of thoracic aortic diameters in the elderly. The Rotterdam Study.

Accepted to poster presentation in European Society Cardiology Congress 2018

Abstract Published in: P4548 Diameters of the thoracic aorta: Gender-specific references ranges and association with body size and atherosclerotic factors European Heart Journal, Volume 39, Issue suppl_1, 1 August 2018, ehy563.P4548, https://doi.org/10.1093/eurheartj/ehy563.P4548

Full text published in BMJ Heart. Heart 2020;106:133–139. pii: heartjnl-2019-315320. doi: 10.1136/heartjnl-2019-315320. [Epub ahead of print]. PMID: 31551294.

Editorial comment published by Groepenhoff F, den Ruijter HM. BMJ Heart 2019;0:1-2. doi:10.1136/heartjnl-2019-315903

Abstract

Objective: To provide population-based distributions of thoracic aortic diameters in men and women aged 55 years or older and to identify determinants of thoracic aortic diameters.

Methods: From 2003 to 2006, 2505 participants (1208 men, mean age 69.1±6.8 years) from the prospective population-based Rotterdam Study underwent non-enhanced cardiac CT. The diameter of the ascending (AA) and descending aorta (DA) was measured at the level of the pulmonary bifurcation.

Results: The mean diameter of the ascending and descending aorta was substantially larger in men (38±4 mm and 30±2 mm) than in women (35±3 mm and 27±2 mm). An ascending aortic diameter of larger than 40 mm was found in 228 (18.9%) men and 76 (5.9%) women and a descending aortic diameter larger than 40 mm was found in two men and no women. Male sex was found to be independently associated with larger DA diameter (standardized ß 0.24, CI 0.19;0.30), while a statistically non-significant trend was found for the AA diameter (standardized ß 0.06, CI 0.00;0.12). Age, height, weight and traditional cardiovascular risk factors were also associated with larger AA and/or DA diameters. Diabetes was associated with smaller AA and DA diameters. We found no evidence for effect modification by sex.

Conclusions: In persons aged 55 years or older, an ascending aortic diameter of 40 mm or larger was found in 18.9% of men and 5.9% of women. Given the importance of sex, sex-specific distribution values may prove useful in clinical practice, even when correcting for BSA or height.

Keywords

Thoracic aorta, aortic reference diameters, computed tomography, population-based research, elderly, determinants.

Key questions

What is already known about this subject?

While aortic diameters change with age and most aortic complications due to aneurysmatic aortic diameters occur at older age, data about the distribution of aortic diameters among older persons are scarce.

What does this study add?

In our population-based cohort, the mean ascending and descending aortic diameters were 38mm and 30mm in men, and 35mm and 27mm in women. In 18.9% of the male participants and 5.9% of the female participants the ascending aortic diameter was larger than 40mm. Most important determinants of thoracic aortic diameters were age, sex, body measures and traditional cardiovascular risk factors.

How might this impact on clinical practice?

In middle-aged and elderly persons the prevalence of an ascending aortic diameter of more than 40mm is relatively high. Further research is needed to assess whether a higher cut-off value for aortic dilatation should be applied for elderly.

Introduction

Individuals with a thoracic aorta dilatation of larger than 60 mm are at high risk for severe complications, such as aortic dissection and rupture,[1] which are related to mortality rates of up to 50% in the acute phase. These serious consequences have led to the development of the current guidelines stating that patients with an absolute thoracic aortic diameter of 55 mm or larger qualify for preventive aortic surgery.[2, 3] Yet, the definition of the threshold for aortic dilatation remains a topic of debate. Some argue that the absolute diameter provides a better risk-estimate than diameters corrected for height and weight, while others propose to correct for body measurements such as body surface area (BSA),[4] especially when it concerns women with short height. The current guidelines from the European Society of Cardiology (ESC)[2] define aortic dilatation as an absolute aortic diameter of larger than 40 mm. When aortic dilatation is found, follow-up visits are recommended to

identify patients who will reach the threshold for preventive surgery due to further aortic growth. An important note regarding this definition of aortic dilatation is that this threshold is derived from cohort-data of relatively young individuals with an age range from 9 to 59 years.[5] Hence, this threshold of 40 mm [6] may not be directly applicable to older individuals, especially given increasing evidence suggesting that aortic diameters change with age.[7-9] Together with the finding that aortic complications mostly occur at older age,[10] this emphasizes the need for data on the distribution of aortic diameters among older persons.[11]

In addition, more insight is required into the existence of sex-specific thoracic aortic diameters, as well as into which determinants influence thoracic aortic diameters. These topics are crucial for further research into the clinical relevance of aortic diameters in the elderly and may even contribute to the development of sex-specific recommendations. Against this background, we investigated (1) sex-specific distributions of absolute and BSA-corrected thoracic aortic diameters in a large sample of middle-aged and elderly persons from the general population, and (2) examined determinants of thoracic aortic diameters.

Methods

Study population

The Rotterdam Study is a prospective population-based cohort study that started in 1990, initially including participants aged 55 years or older from the Ommoord district in Rotterdam.[12] Between 2003 and 2006, a random sample of 2524 participants underwent non-enhanced multidetector CT as part of a large project on arterial calcification. We excluded 17 CT-examinations due to image artefacts because of the presence of pacemakers or coronary stent implantations (n=5), poor image acquisition quality (n=4), or absence of the pulmonary artery bifurcation level in the images (n=8). From two participants with complete data of aortic measurements, cardiovascular risk factor assessment at baseline was lacking. The remaining 2505 persons form the basis of the current analyses. In these 2505 participants, the ascending aortic diameter could be measured in 2500 (99.8%) and the descending aorta in 2462 (98.3%) participants. The Rotterdam Study complies with the Declaration of Helsinki and has been approved by the medical ethics committee, according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Participants were not involved in the design and conduct of the current research.

Assessment of aortic diameters

Non-contrast CT images were obtained using 16-slice (n=775) or 64-slice (n=1730) multidetector CT scanners (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany). Two scans were obtained: A prospective ECG triggered cardiac scan started at the apex of the heart and ended at the tracheal bifurcation and an extra-cardiac scan that reached from the aortic arch to the intracranial vasculature. Aortic diameters were measured primarily on the cardiac scan at an R-R interval of 50%. When the ascending or descending aorta was not visible in the heart scan, the extra-cardiac scan was used (ascending aorta n=162 and descending aorta n=173). Detailed information on the scan protocol has been provided previously.[13] Both the ascending and descending aortic diameters were measured in millimetres in two directions at the bifurcation level of the pulmonary artery using the double-oblique method in a reconstruction perpendicular to the vessel axis. The largest diameter of the two measurements was used for further analysis. Given the lack of contrast material, we measured the aortic diameter with the outer edge-to-outer edge method. Assuming that calcified plaques are located in the intimal layer of the aorta, they were included in the measurement. The aortic diameters were measured by three observers. To assess interobserver variability each observer measured the aortic diameters of the first 100 participants. The intraclass correlation coefficient was 0.985 for the ascending aorta and 0.989 for the descending aorta. Mean differences between the three observers were determined by Bland-Altman plots (supplemental figure 1).[14] Since body size area (BSA) adjusted values are introduced by others,[4] we also presented the diameter adjusted for BSA. BSA was calculated using the Dubois and Dubois formula: BSA (m^2) = 0.007184 x Height(m)$^{0.725}$ x Weight(kg)$^{0.425}$.

Assessment of determinants

We gathered information on the following determinants: age, body measurements, systolic and diastolic blood pressure, smoking, alcohol consumption, coronary and aortic arch calcification volume, cholesterol levels, diabetes, history of cardiovascular disease (CVD) and medication use. Detailed information on the assessment strategy for each determinant is provided in the Appendix A.

Mortality due to aortic events during follow-up

For all Rotterdam Study participants, municipal records were checked for information on vital status. Mortality due to an aortic event (aortic aneurysm or

dissection) is collected according to the WHO including ICD-10 code I71 and was available until 1 January, 2014.

Statistical analysis

Continuous variable are expressed as mean ± SD or as median ± IQR. Data distribution was checked using histograms and the Shapiro-Wilk test. Categorical variables are presented as frequencies with percentages. We calculated the sex-specific distributions with mean and 95th percentile of absolute and BSA-adjusted thoracic aortic diameters for the total group and for different age groups (55-64 years, 65-74 years and ≥75 years). As sensitivity analysis, we excluded the participants with a history of CVD.

Associations between the determinants and absolute ascending or descending aortic diameters were quantified with univariable and multivariable linear regression models. We certified that the following assumptions underlying linear regression were met: linearity and homoscedasticity with a plot of *ZRESID against *ZPRED, normal distribution of residuals with histograms and normal P–P plots, multicollinearity with the variance inflation

factor (VIF) and independent errors with the Durbin-Watson test. In multivariable linear regression analyses, all determinants (ie, age, anthropometrics, sex, smoking, alcohol consumption, diabetes, blood pressure, medication use, lipids, calcification volumes, and history of CVD) were included in the model. For the analyses of calcification volumes, we used natural log-transformed values and added 1.0 mm^3 to the non-transformed calcification values (ln(calcification volume + 1)) to deal with calcium volumes of zero. In order to reduce the effect of multicollinearity, we removed a variable when a VIF of more than 10 was found. All models were adjusted for cohort and scanner type. We investigated statistical interaction of sex on all associations of determinants with the aortic diameters by adding interaction terms (sex x determinant) to the models. In addition, we determined to what extent only age and sex and age, sex and anthropometrics explained the variance in ascending and descending aortic diameters.

In 12.7% of the participants, ≥1 of the covariates were missing and they were handled by multiple imputation with five iterations.[15] IBM SPSS statistics software V.21.0 was used to analyse the data and a p-value of <0.05 was considered statistically significant.

Results

Study population

The median age of the 2505 participants was 67 (IQR 64-73) years, and 51.8% were women (table 1). CVD was prevalent in 303 participants (12%).

Sex-specific distributions of thoracic aortic diameters

Sex-specific distributions of absolute and BSA-adjusted aortic diameters are given for the total group and for different age groups in table 2. For the ascending and descending thoracic aorta, the mean diameters in men were 38±4 mm and 30±2 mm, and 35±3 mm and 27±2 mm in women. The full distribution of the absolute diameters, including the 90th and 95th percentiles, are shown in figure 1. After exclusion of participants with a history of CVD, the results did not substantially change. An ascending aortic diameter larger than 40 mm was found in 228/1208 men (18.9%) and in 76/1292 women (5.9%). An aortic diameter larger than 45 mm was found in 26/1208 men (2.2%) and 7/1292 women (0.5%), among whom 4 (0.2%, 3 men) had a diameter larger than 50 mm. A descending aortic diameter of larger than 35 mm was found in 20/1169 (1.7%) men and 4/1293 women (0.3%), and larger than 40 mm in 2 (0.2%) men and no women. None of the participants had a descending diameter of 45 mm or larger. From the 304 participants (12.2%) with an aortic diameter of more than 40 mm, only 4 (1.3%, 3 men and 1 woman) participants died of an aortic event (supplemental table 1).

Determinants of ascending and descending aortic diameters

The results for invariable analyses are presented in supplemental table 2 and the univariable associations of height, weight and BSA with aortic diameters are visualised in supplemental figure 2. Multivariable linear regression analysis showed that higher age, taller height and larger weight, higher diastolic blood pressure, lower systolic blood pressure, larger volume of calcifications in coronary arteries and aortic arch and the use of blood pressure-lowering medication were associated with larger absolute ascending and descending aortic diameters (figures 2 and 3). Conversely, the presence of diabetes and the use of lipid-modifying agents were associated with smaller ascending and descending aortic diameters. A smaller hip circumference was specifically associated with a smaller ascending aortic diameter. Male sex, current smoking, alcohol consumption and lower high-density

lipoprotein (HDL) cholesterol were specifically associated with larger descending aortic diameters. None of the interaction terms between the potential determinants and sex was significant. Age, sex and anthropometrics explained 15% of the variance in ascending aortic diameters while age and sex explained 34% of the variance in descending thoracic aortic diameters. Addition of anthropometrics and conventional cardiovascular risk factors increased this to 21% for the ascending aorta and to 39% for the descending aorta.

Discussion

Using data from a large population-based cohort, we provide new sex-specific distributions of absolute and BSA-corrected thoracic aortic diameters in middle aged and elderly persons. For the ascending and descending aorta, the mean diameters in men were 38 mm and 30 mm, and 35 mm and 27 mm in women. An ascending aortic diameter larger than 40 mm was found in 304 participants (12.2%, 228 men) and a descending aortic diameter larger than 40 mm was found in two participants. Although we found a thoracic aortic diameter of more than 40 in a considerable amount of persons aged 55 years or older, only 4 (1.3%) of them died as a result of an aortic event. This number seems rather low and raises the question whether a cut-off of 40 mm is an appropriate one. Yet, given the low number of events, this should be confirmed by larger studies.

Although the difference in mean aortic diameter between the age group of 55-65 years and ≥75 years was only 1 mm for both the ascending and descending aorta, the aortic diameter above 55 years still increased. Our results show the 95th percentile for persons above the age of 75 years old to be 43 mm for ascending and 35 mm for descending aorta for men and 41 mm and 33 for women, an age group for whom distribution data are currently scarce.[11] Our data further establish the range of thoracic aortic diameters, which is similar to data found in a study performed in Germany.[16] Absolute aortic diameters measured in the Rotterdam Study are larger than previous studies performed in the USA,[9, 17] which also used non-enhanced CT imaging. They reported older participants (≥55 years) as subanalysis of the entire population studied and therefore contained smaller samples. The larger aortic diameters measured in our study may partly be explained by the larger average height of the study population. Native Dutch people are relatively tall.[18] BSA of our cohort (1.9±0.2 m^2) was comparable with the aforementioned two studies (both 1.9±0.3 m^2), but height was not reported by others and therefore could not be compared. BSA describes height and also weight, and since American individuals are more likely to be obese than Dutch individuals[19] this might suggest a taller

height in our group. Nevertheless, both our cohorts as previously smaller cohorts show that an aortic diameter of >40 mm is not uncommon in middle-aged and elderly persons.

In our cohort, both weight and height were found to be important determinants of the aortic diameters, which supports previous findings on this matter.[7, 8] Whether the aortic diameter should be corrected for height, weight or BSA in defining aortic dilatation is an ongoing discussion. While some authors suggest to use only height,[20] others advocate the use of BSA, which takes into account both height and weight.[4] However, longitudinal data are needed to establish the abilities of indexed aortic diameters compared with the abilities of absolute aortic diameters in the prediction of aortic events. Interestingly, from our data, it appeared that absolute values are substantially larger for men than for women, yet BSA-corrected values are statistically larger for women than for men. This suggest that differences in body measures partly explain sex differences in aortic diameters, but that there is still a remaining sex-difference which results in a larger BSA-indexed values for women. Therefore, we conclude that distribution values should be provided for men and women separately, even when correcting for weight, height or BSA.

In line with previous literature,[9, 17, 21] smoking was associated with the diameter of the descending but not the ascending aorta. In addition, HDL cholesterol had a significant inverse relation with only the descending aorta. Based on figure 2 and 3, 39% of the variance in descending aortic diameter but only 21% of the variance in ascending aortic diameter was explained by age, sex, anthropometrics and traditional cardiovascular risk factors. Probably, other factors such as genetic predisposition are more important in the ascending aorta. Already 34% of the variation in descending aortic diameter and 15% of the variation in ascending aortic diameter was explained by age, sex and anthropometrics. Therefore there is only a small increase in explained variance caused by the addition of traditional cardiovascular risk factors to the model. Since this small increase in explained variance was shown in both the ascending and descending aorta, we provide no clear evidence that the descending aorta is more susceptible to cardiovascular risk factors than the ascending aorta. Both the ascending and descending aortic diameter were associated with calcifications of the coronary arteries or aortic arch and use of lipid-modifying agents. This is in contrast to recent literature showing a significant relation of aortic plaques and calcium with only the descending aorta.[21, 22] In conclusion, although we found differences between the ascending and descending aorta in the association between smoking, alcohol consumption and HDL cholesterol, the effect of these cardiovascular risk factors seems of limited importance in explaining the overall variation in aortic diameter.

In addition, we found that blood pressure was associated with thoracic aortic diameters. We found a positive association of the diastolic blood pressure, but a negative association of the systolic blood pressure with aortic diameters. Whether hypertension is indeed a risk factor for aortic dilatation is still unknown,[23] but our results suggest that high diastolic blood pressure might be more important in the development of aortic dilatation than high systolic blood pressure. Current guidelines[2] advise to reduce blood pressure (both systolic and diastolic). The importance of high diastolic blood pressure on aortic diameters should be stressed and deserves more attention, also in research.

We found diabetes to be negatively associated with both the ascending and descending aortic diameters. This phenomenon has already been shown in the abdominal aorta, where it is described that diabetes is associated with less aortic dilatation of the abdominal aorta.[24, 25] This might be caused by advanced glycation associated with diabetes which inhibits through intermediate steps secretion of the matrix metalloproteinases.[25] Also, the fibrinolytic pathway, more specifically the plasminogen activator inhibitor-1, is mentioned as a candidate mechanism for hyperglycaemic inhibition of abdominal aortic disease.[26] More research is warranted to elucidate the role of these pathways in the development of both thoracic and abdominal aortic aneurysms.

Strengths of our study include the population-based setting and the relatively large sample size. By including participants with hypertension or a history of CVD, we measure the aortic diameter in the general population and not only in healthy people. Therefore the results can be generalised to a larger proportion of the older population.

Limitations

The use of ECG-gated CT scans allowed highly accurate measurements of the aorta. The use of contrast-enhanced CT would have made the measurements even more accurate. However, the use of contrast in predominantly healthy people from the general population is unethical and can cause unnecessary complications, such as an allergic reaction to contrast fluid.

Conclusion

We provide novel sex-specific distributions of thoracic aortic diameter for the middle-aged and elderly general population. Our distribution show high prevalence

(12.2%) of an ascending aortic diameter more than 40 mm, which is typically considered dilated. However, mortality due to aortic aneurysm or dissection in these participants seems rather low, and raises the question whether a cut-off of 40 mm is an appropriate one. Yet, given the low number of events, this should be confirmed by larger studies.

Sex was independently associated with descending aortic diameters and tended to be associated with ascending aortic diameters. This indicates that distribution values should be provided for men and women separately, even when correcting for BSA or height. Traditional cardiovascular risk factors are responsible for only a limited part of the variance in aortic diameters. We found no evidence for effect modification of these associations by sex.

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Table 1. Baseline Characteristics of Study Participants

Variables	Total (n=2505)	Men (n=1208)	Women (n=1297)
Age (years)	67 (64-73)	68 (64-73)	67 (64-73)
Height (cm)	168±9.5	175±7	161.5±6.3
Weight (kg)	78.4±13.7	84±12	73.0±12.8
Body surface area (m^2)	1.9±0.2	2.0±0.2	1.8±0.2
Hip circumference (cm)	103±8	102±6	105±9
Systolic blood pressure (mmHg)	147±20	146±20	147±21
Diastolic blood pressure (mmHg)	80±11	81±11	79±11
Smoking			
Never (%)	718 (29%)	188 (16%)	530 (41%)
Past (%)	1363 (54%)	796 (66%)	567 (44%)
Current (%)	424 (17%)	224 (19%)	200 (15%)
Alcohol consumption			
Never (%)	154 (6%)	30 (3%)	124 (10%)
Past (%)	182 (7%)	83 (7%)	99 (8%)
Current (%)	2169 (87%)	1095 (91%)	1074 (83%)
Coronary calcification volume (mm^3)*	5 (2-287)	138 (20-490)	18.8 (0-125)
Aortic arch calcification volume (mm^3)*	267 (49-881)	297 (55-1010)	238 (43-819)
Total cholesterol (mmol/L)	5.7±1.0	5.4±1.0	5.9±1.0
HDL cholesterol (mmol/L)	1.4±0.4	1.3±0.3	1.6±0.4
Diabetes mellitus (%)	338 (14%)	176 (15%)	162 (13%)
History of cardiovascular disease	303 (12%)	210 (17%)	93 (7%)
Myocardial infarction (%)	143 (6%)	103 (9%)	40 (3%)
PCI (%)	80 (3%)	60 (5%)	20 (2%)
CABG (%)	89 (4%)	77 (6%)	12 (1%)
Stroke (%)	102 (4%)	59 (5%)	43 (3%)
Medication			
Blood pressure lowering medication (%)	1046 (42%)	522 (43%)	524 (40%)
Antithrombotic agents (%)	625 (25%)	310 (26%)	315 (24%)
Serum lipid reducing agents (%)	608 (24%)	365 (30%)	243 (19%)

Values are presented as mean (SD) or median (IQR) for continuous variables and N (%) for categorical variables. Data represent imputed values. Missing values were present for height and weight (0.8%), hip circumference (0.7%), blood pressure (0.4%), smoking and alcohol consumption (2.9%), calcification in coronary arteries

(1.4%), calcification in aortic arch (0.2%), serum lipids (1.6%), diabetes mellitus (0.4%), lipid-modifying agents (1.5%) and blood pressure lowering medication (1.5%). HDL indicates high-density lipoprotein. *Nontransformed median volume with interquartile range.

Table 2. Distribution of absolute aortic diameter and adjusted for BSA.

			Ascending aorta			Descending aorta		
	Age group		**Total**	**Men**	**Women**	**Total**	**Men**	**Women**
Absolute diameter (mm)	Total	Number	2500	1208	1292	2462	1169	1293
		Mean	36±4	38±4	35±3	29±3	30±2	27±2
		95th	43	44	41	33	34	31
	55-64 years	Number	724	341	383	721	335	386
		Mean	36±4	37±4	35±3	28±3	29±2	27±2
		95th	42	44	40	32	33	30
	65-74 years	Number	1238	610	628	1217	590	627
		Mean	37±4	38±4	35±3	29±3	30±2	27±2
		95th	43	44	41	33	34	31
	≥75 years	Number	538	257	281	524	244	280
		Mean	37±4	38±4	36±3	29±3	31±3	28±2
		95th	43	43	41	34	35	33
BSA-indexed diameter (mm/m2)	Total	Number	2500	1208	1292	2462	1169	1293
		Mean	20±2	19±2	20±2	15±2	15±2	16±2
		95th	24	22	24	18	18	19
	55-64 years	Number	724	341	383	721	335	386
		Mean	19±2	18±2	20±2	15±1	14±1	15±1
		95th	23	22	23	17	17	17
	65-74 years	Number	1238	610	628	1217	590	627
		Mean	19±2	19±2	20±2	15±1	15±1	15±1
		95th	24	23	24	18	18	18
	≥75 years	Number	538	257	281	524	244	280
		Mean	20±2	19±2	21±2	16±2	16±2	17±2
		95th	25	23	25	19	18	20

Mean value is given with the standard deviation.

Figure 1. Distribution of the ascending and descending aortic diameters for both men (blue) and women (red), marking mean values, the 90th and 95th percentiles.

Figure 2. Determinants of ascending aortic diameters in multivariable analysis.
Models were further adjusted for cohort and scanner type. The variance of the ascending aortic diameter was explained by conventional cardiovascular risk factors for 21%.
95% CI= 95% confidence interval.
CVD = cardiovascular disease
* p-value <0.05
† Values represents transformed calcification volumes in mm^3: Ln(calcification volume+1 mm^3)

Figure 3. Determinants of descending aortic diameters in multivariable analysis.
Models were further adjusted for cohort and scanner type. The variance of the descending aortic diameter was explained by conventional cardiovascular risk factors for 39%.
95% CI= 95% confidence interval.
CVD = cardiovascular disease
* p-value <0.05
† Values represents transformed calcification volumes in mm^3: Ln(calcification volume+1 mm^3)

Figure 1

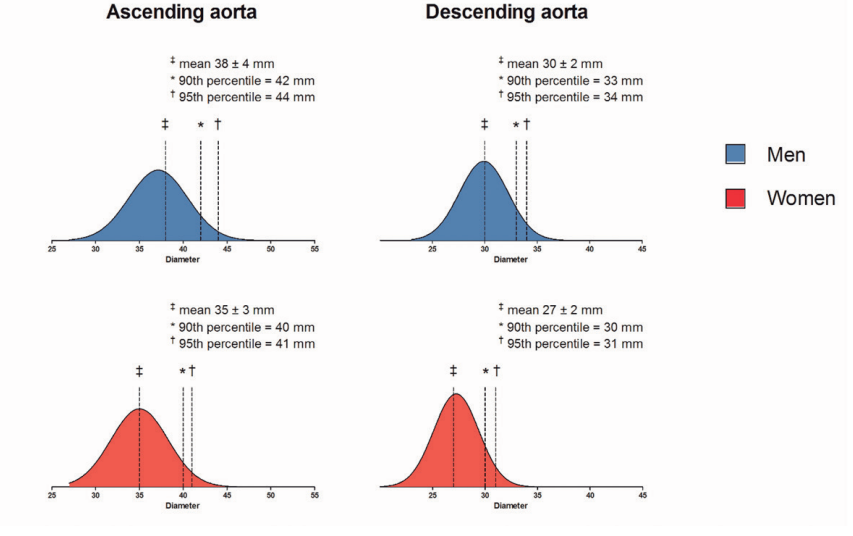


Figure 2

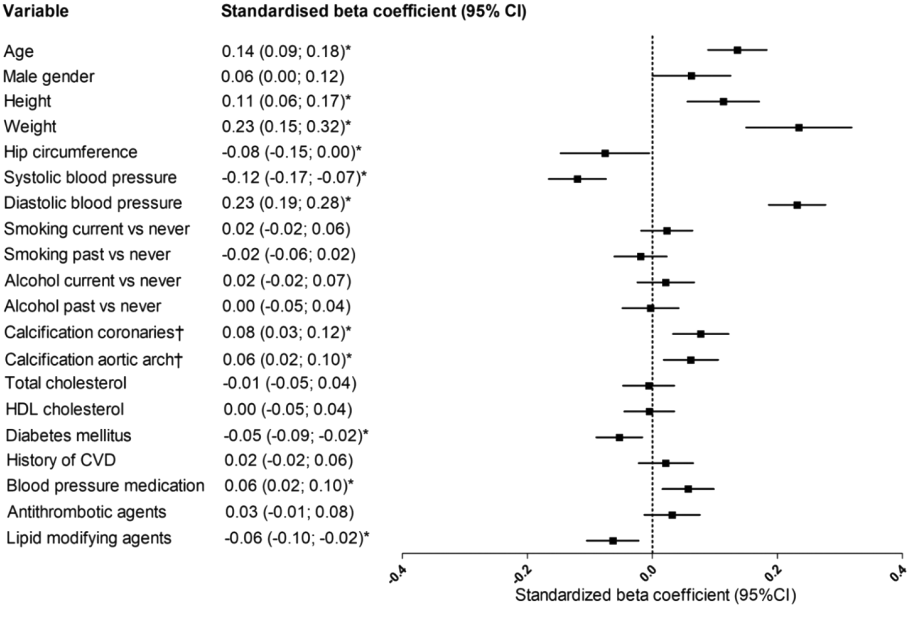
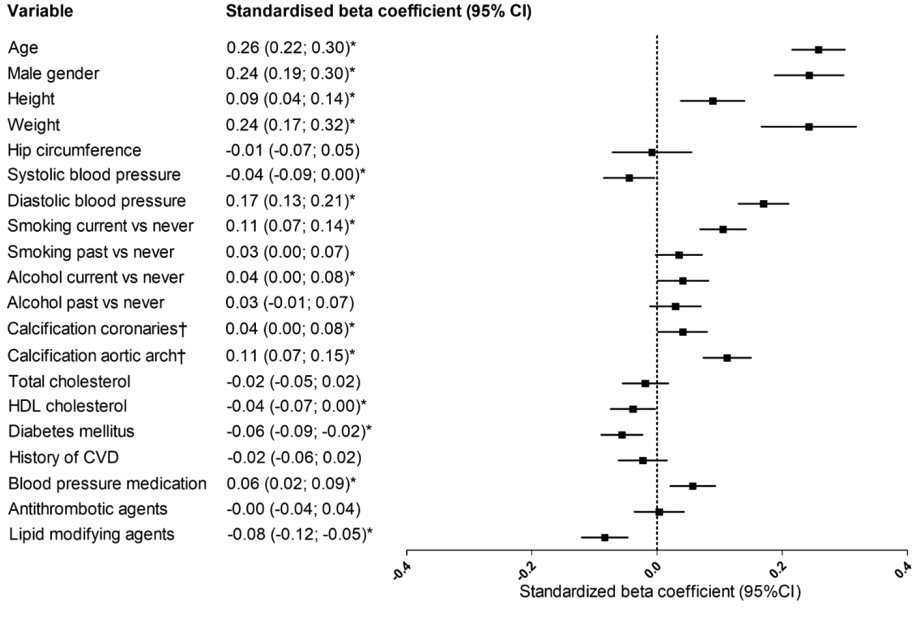


Figure 3



SUPPLEMENTAL MATERIAL

Bons LR, Rueda-Ochoa OL, K El Ghoul, et al. Sex-specific distributions and determinants of thoracic aortic diameters in the elderly.

Appendix A. Assessment of determinants

eTable 1. Characteristics of participants who died due to aortic aneurysm or dissection during follow-up

eTable 2. Univariable analysis for ascending and descending aortic diameters stratified by sex

eFigure 1. Bland-Altman agreement between three observers

eFigure 2. Mean aortic diameter of the ascending and descending aorta plotted against height, weight and body surface area in men and women separately.

Assessment of determinants

Data on current and former smoking habits and alcohol consumption were collected during a structured home interview. We assessed information on the use of blood pressure lowering medication, antithrombotic agents and lipid modifying agents including statins according to the ATC/DDD system (http://www.whocc.no). Clinical measurements were collected during regular visits at the research center. (1) Height, weight, waist and hip circumference were assessed. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The average of three consecutive measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg and/or the use of blood pressure-lowering medication. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using an automated procedure. Diabetes was defined as the use of glucose-lowering medication and/or a fasting glucose level ≥7 mmol/L. Coronary artery calcification (CAC) and aortic arch calcification (AAC) volumes were quantified using commercially available software (Syngo CalciumScoring; Siemens).(2) A history of cardiovascular diseases (myocardial infarction, percutaneous coronary intervention, coronary revascularization, and stroke) was ascertained as described in detail previously.(3)

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						Ascending aorta				Descending aorta			
Participant	**ICD-code***	**Gender**	**Age (years)**	**Height (cm)**	**Weight (kg)**	**Crude diameter**	**Height-indexed**	**Weight-indexed**	**BSA-indexed**	**Crude diameter**	**Height-indexed**	**Weight-indexed**	**BSA-indexed**
1	I71.1	Men	71	190.60	93.40	47	24.7	0.50	21.2	33	17.3	0.35	14.9
2	I71.1	Women	81	159.50	67.40	43	27.0	0.64	25.3	32	20.1	0.47	18.8
3	I71.2	Women	82	161.20	74.38	38	23.6	0.51	21.3	35	21.7	0.47	19.6
4	I71.3	Women	67	157.00	69.80	37	23.6	0.53	21.7	34	21.7	0.49	19.9
5	I71.4	Women	60	161.50	49.50	39	24.2	0.79	25.9	32	19.8	0.65	21.3
6	I71.8	Men	60	174.80	98.20	42	24.0	0.43	19.7	36	20.6	0.37	16.9
7	I71.8	Men	78	177.00	71.90	41	23.2	0.57	21.8	36	20.3	0.50	19.1
8	I71.8	Men	85	173.00	82.80	39	22.5	0.47	19.8	33	19.1	0.40	16.8

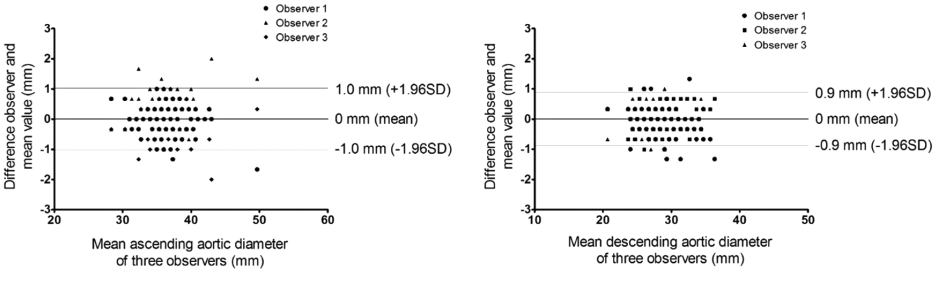
eTable 1. Characteristics of participants who died due to aortic aneurysm or dissection during follow-up

* I71.1 thoracic aortic aneurysm, ruptured, I71.2 thoracic aortic aneurysm, without mention of rupture, I71.3 abdominal aortic aneurysm, ruptured, I71.4 abdominal aortic aneurysm, without mention of rupture, I71.8 aortic aneurysm of unspecified site, ruptur

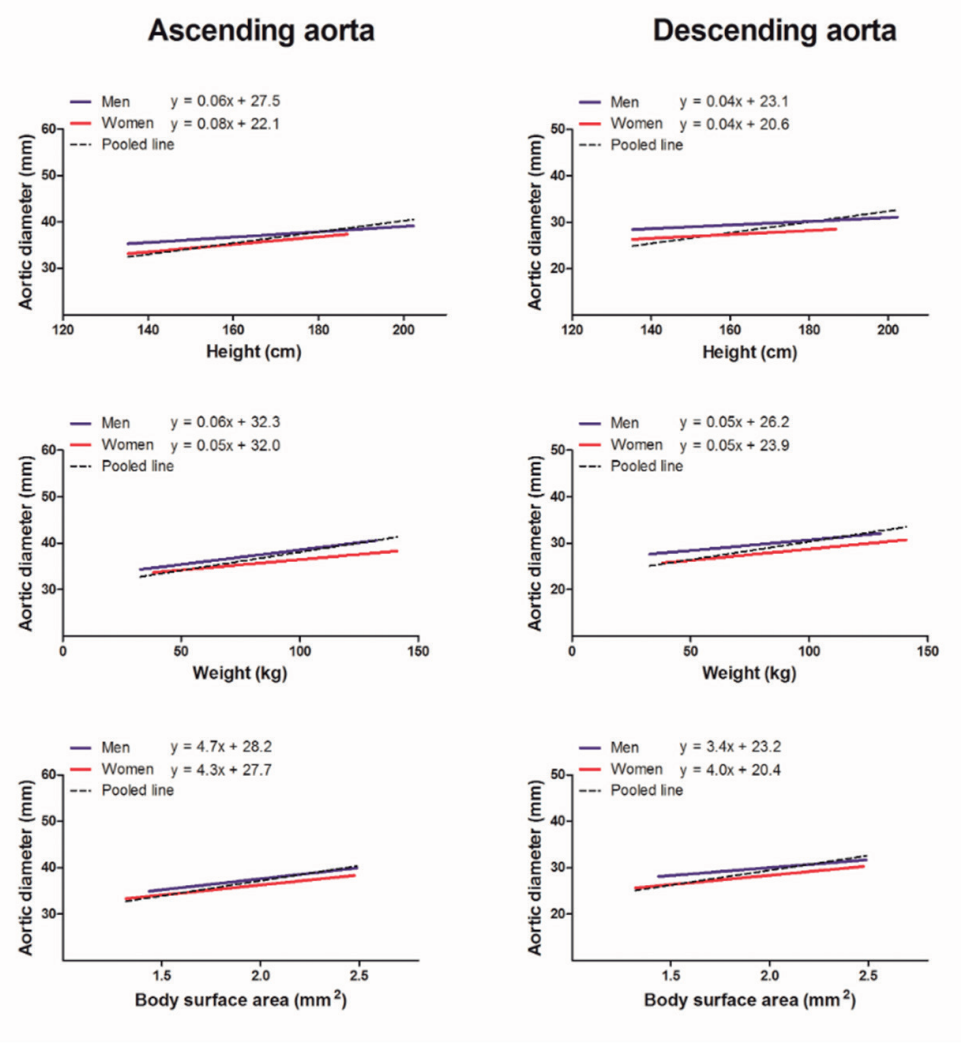
Variables	Ascending aorta Beta (95% CI)	Descending aorta Beta (95% CI)
Age (years)	0.04 (0.02; 0.06)*	0.08 (0.07; 0.10)*
Height (cm)	0.12 (0.11; 0.13)*	0.12 (0.11; 0.13)*
Weight (kg)	0.08 (0.07; 0.09)*	0.08 (0.07; 0.08)*
Hip circumference (cm)	0.04 (0.02; 0.05)*	0.03 (0.02; 0.05)*
Systolic blood pressure	0.01 (0.00; 0.01)*	0.02 (0.01; 0.02)*
Diastolic blood pressure	0.06 (0.05; 0.08)*	0.04 (0.03; 0.05)*
Smoking		
Past versus never	0.57 (0.28; 0.86)*	0.76 (0.55; 0.97)*
Current versus never	0.35 (-0.04; 0.73)	0.60 (0.32; 0.89)*
Alcohol consumption		
Past versus never	-0.60 (-1.23; 0.4)	-0.15 (-0.63; 0.33)
Current versus never	0.88 (0.44; 1.33)*	0.77 (0.43; 1.10)*
Calcification volumes in coronary artery	0.31 (0.25; 0.36)*	0.30 (0.26; 0.34)*
Calcification volumes in aortic arch	0.18 (0.12; 0.24)*	0.25 (0.21; 0.30)*
Total cholesterol (mmol/L)	-0.35 (-0.49; -0.20)*	-0.41 (-0.51; -0.30)*
HDL-cholesterol (mmol/L)	-1.48 (-1.84; -1.12)*	-1.74 (-2.00; -1.47)*
Diabetes mellitus	-0.20 (-0.62; 0.22)*	0.07 (-0.25; 0.38)
History of cardiovascular disease	1.08 (0.64; 1.52)*	0.74 (0.41; 1.07)*
Blood pressure lowering medication	0.83 (0.54; 1.12)*	0.74 (0.53; 0.96)*
Antithrombotic agents	0.90 (0.57; 1.23)*	0.71 (0.46; 0.95)*
Serum lipid reducing agents	-0.05 (-0.38; 0.28)	-0.15 (-0.40; 0.09)

eTable 2. Univariable analysis for ascending and descending aortic diameters
95% CI= 95% confidence interval.
* p-value <0.05.

eFigure 1



eFigure 2


Ascending aorta
Descending aorta
Men y = 0.06x + 27.5
Women y = 0.08x + 22.1
Pooled line
Aortic diameter (mm)
Height (cm)
Men y = 0.04x + 23.1
Women y = 0.04x + 20.6
Pooled line
Aortic diameter (mm)
Height (cm)
Men y = 0.06x + 32.3
Women y = 0.05x + 32.0
Pooled line
Aortic diameter (mm)
Weight (kg)
Men y = 0.05x + 26.2
Women y = 0.05x + 23.9
Pooled line
Aortic diameter (mm)
Weight (kg)
Men y = 4.7x + 28.2
Women y = 4.3x + 27.7
Pooled line
Aortic diameter (mm)
Body surface area (mm2)
Men y = 3.4x + 23.2
Women y = 4.0x + 20.4
Pooled line
Aortic diameter (mm)
Body surface area (mm2)

CHAPTER 4.2

Thoracic aortic diameters and major adverse cardiovascular outcomes: The Rotterdam Study.

Moderated poster presentation European Society Cardiology Congress 2018
Published in:
P6039 Diameters of the thoracic aorta and their association with mortality in the general population. *European Heart Journal*, Volume 39, Issue suppl_1, 1 August 2018, ehy566.P6039, https://doi.org/10.1093/eurheartj/ehy566.P6039

Poster presentation European Society Cardiology Congress 2019
Published in:
P1818 Descending aortic thoracic diameter: a risk marker for major adverse cardiovascular outcomes in women. *European Heart Journal*, Volume 40, Issue suppl_1, October 2019,

Full text submitted to European Journal of Preventive Cardiology 2020.

Abstract

Objective: To assess the independent association between crude and indexed ascending and descending aortic (AA and DA) diameters with major cardiovascular outcomes among women and men.

Methods: 2178 women and men ≥55 years underwent multi-detector CT scan of thorax from the Rotterdam study, during 13 years of follow-up. Crude and indexed diameters of the AA and DA, and dichotomous cutoff points (corresponding to 75th to 95th percentiles) were measured. Incidence of stroke, coronary heart disease (CHD), heart failure (HF), cardiovascular and all-cause mortality were outcomes under study.

Results: Among women, larger crude and almost all indexed DA diameters showed significant associations with the outcomes (hazard ratios-HRs (95%CI) per 1 standard deviation-SD increase in crude DA diameter) were 1.38 (1.03, 1.84) for stroke, 1.33 (1.01, 1.74) for HF, 1.47 (1.05, 2.05) for cardiovascular mortality, and 1.20 (1.02, 1.40) for total mortality. Among men, only weight-indexed DA diameters were associated with total mortality: 1.47 (1.21, 1.77). For AA, larger weight-indexed values were significantly associated with higher risk of mortality in both sexes: 1.19 (1.05, 1.36) in women and 1.38 (1.16, 1.65) in men). For crude DA diameter, the 75th percentile showed significant association with incident stroke among men while the 75th and 85th percentiles showed significant associations with incident HF and cardiovascular mortality respectively in women.

Conclusions: Our study suggests a role for descending aortic diameter as a marker for increased cardiovascular risk, in particular among women.

Keywords: Thoracic aortic diameters, Indexed diameters, Cohort Study, Cardiovascular outcomes, sex-differences.

What is already known on this subject?

Changes of vessel size and structure (i.e. vascular remodeling), increases with age and have a systemic nature involving hemodynamic and biological processes (oxidation, inflammation, matrix degradation, fibrosis) that play a key role in cardiovascular disease (CVD) pathogenesis.

What might this study add?

While enlargement of the thoracic aorta is a frequent finding in clinical practice, few longitudinal data regarding its long-term prognosis for major CVD outcomes at population level, other than rupture or dissection, exist.

How might this impact on clinical practice?

Our study suggests gender differences in the role for aortic diameter as a marker for increased cardiovascular risk, in particular among women. The cutoff points for increased risk for several of cardiovascular outcomes were below the 95th percentile of the distribution of aortic diameters. This implies that not only patients with aortic dilatation (aortic diameter >95th percentile) are at risk for major adverse outcomes, but a large group of individuals with mildly enlarged aortic diameters in the general population are at increased risk for cardiovascular outcomes and mortality.

Introduction

Dilatation of thoracic aorta has a silent nature. The risk of natural complications, including dissection or rupture, rises with increasing aortic diameter[1]. Therefore, indications for surgical interventions, recommended by the international guidelines, are mainly based on aortic diameter and derived from weighting the risk of potential natural complications against the risk of elective surgery[2,3]. However, aortic diameter also increases with age. Changes of vessel size and structure (i.e. vascular remodeling), have a systemic nature involving hemodynamic and biological processes (oxidation, inflammation, matrix degradation, fibrosis)[4] that play a key role in cardiovascular disease (CVD) pathogenesis. While enlargement of the thoracic aorta is a frequent finding in clinical practice, few longitudinal data regarding its long-term prognosis for major CVD outcomes at population level, other than rupture or dissection, exist[5].

The limited available longitudinal data focuses mainly on the aortic root and suggest an association between aortic root diameter with CVD and mortality[5]. The association between ascending or descending aorta and cardiovascular events has only been investigated in two studies.[6,7] Additionally, data regarding the sex-specific long-term prognostic value of dilatation of different segments of thoracic aorta for different cardiovascular outcomes are lacking. As the aortic diameter is significantly related to body measurements[8], use of aortic diameters indexed for body measurements could improve its prognostic value for cardiovascular outcomes. Assessing the cardiovascular risk associated with thoracic aortic size among asymptomatic women and men could lead to effective, sex-specific, prevention strategies.

Within the large population-based Rotterdam Study[9], we evaluated the association between computed tomography (CT)-assessed ascending and descending thoracic aortic diameters, both absolute and indexed, with cardiovascular events and mortality during 13 years of follow-up among women and men. We further assessed the predictive value of thoracic aortic diameters for cardiovascular events at dichotomous cutoff points corresponding to sex-specific percentiles of thoracic aortic diameters distribution.

Methods

Study Population

The Rotterdam Study (RS) is a prospective population-based cohort including individuals living in the well-defined Ommoord district in the city of Rotterdam, the Netherlands. The study started in 1990 (RS-I) including 7,983 individuals and was extended twice (RS-II and RS-III) to include subjects who turned 55 after the start of the study or migrated into the research area after the start of the study. By the end of 2008, the Rotterdam Study comprised 14,926 subjects aged 45 years or over[9]. Between 2003 and 2006, 2524 participants, who visited the research center for their regular follow-up visits (fourth visit of RS-1 and second visit of RS-II), were invited to undergo non-enhanced multi-detector CT scan of thorax. For this longitudinal analysis, we excluded participants with prevalent stroke, coronary heart disease (CHD) or heart failure (HF) at the time of the CT. This left 2178 participants for the analyses in the current study. All participants provided written informed consent. The Rotterdam study complies with the declaration of Helsinki and has been approved by the medical ethics committee, according to the population screening act: Rotterdam study, executed by the ministry of health, welfare and sports of the Netherlands.

Ascending and descending thoracic aortic diameters

Ascending and descending thoracic aortic diameters were measured in millimeters by non-contrast ECG-gated CT images obtained using 16-slice (n= 642) or 64-slice (n=1536) multidetector CT scanners (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany) by the double-oblique method in two directions at the bifurcation level of the pulmonary artery. A full report of the scan protocol was described in detail earlier[10]. For the current analyses, in addition to the crude aortic diameters, the aortic diameters of all participants were also corrected for height, weight, body mass index (BMI) and body surface area with use of the aortic size index (ASI): ASI (cm/m^2) = aortic diameter (cm) / body surface area (m^2). Body surface area was calculated using the Dubois and Dubois formula[11]: BSA (m^2) = $0.007184 \times Height(m)^{0.725} \times Weight(kg)^{0.425}$. Coronary artery calcification (CAC) and aortic arch calcification (AAC) were quantified using commercially available software (Syngo CalciumScoring; Siemens)[12].

Cardiovascular risk factors

Previous medical history and comorbidities including hypertension, diabetes mellitus, current health status, smoking and alcohol consumption (never, past and current) and use of medications (blood pressure lowering, lipid lowering, and antithrombotic medications) were assessed by a trained interviewer at the home visit using a computerized questionnaire. A detailed explanation of the methods for measurement of the covariables can be found in the Supplementary material.

Cardiovascular events and mortality

Cardiovascular events in this study included stroke, CHD, HF, cardiovascular mortality and all-cause mortality. All Rotterdam Study participants are intensively and constantly followed to identify the development of all adverse outcomes. A detailed explanation of the methods for outcome assessment can be found in the online-only supplemental material.

Statistical analysis

Continuous variables are presented as mean ± standard deviation or as median ± interquartile range (IQR) and categorical variables as numbers (percentages, %). Continuous variables were compared using independent t-test in case of normal

distribution or Mann-Whitney test in case of skewed distribution. Chi-square was used for comparison among categorical variables. Diameters of ascending and descending aorta were indexed by height in meters, weight in kilograms, BSA and BMI. We further standardized the crude and indexed ascending and descending aortic diameters for use in statistical analyses.

A Cox proportional hazard model was used to evaluate the association between time to each major adverse event and each aortic diameter (crude, indexed values). We reported the hazard ratios (HR) with 95% confidence intervals (CI). The analyses were adjusted for age, waist to hip ratio (WHR), smoking, alcohol consumption, total and high-density lipoprotein (HDL) cholesterol, hemoglobin, hypertension, diabetes mellitus, lipid lowering medications, anti-thrombotic medication, and CAC and AAC. Additionally, height and weight were used as covariates in the models that did not include them for indexing the aortic diameters. For each of the final models, proportional hazards assumption was evaluated through Schoenfeld residuals, Goodness-of-fit through linktest and Cox Snell residuals, linearity of covariables through martingales, and influential observations through deviance residuals and Cook distance. As a sensitivity analysis, we further performed competing risk analysis for cardiovascular mortality and non-cardiovascular mortality using non-parametric and semiparametric (Fine and Gray) methods evaluating cause specific hazard and cumulative incidence function.

We further examined associations of crude AA and DA diameters with the outcomes at dichotomous cutoff points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles. The associated discriminative capacity for each cutoff point was evaluated using Harrell's C statistics. We further developed Cox proportional hazard models and examined the associations between percentiles based cutoff points for each crude aortic diameter and the risk for each cardiovascular outcome. These models were adjusted for the same covariates described above.

The analyses were performed with STATA (version 14.0, Stata Corp, College Station, TX). A two-sided P value of <0.05 was considered statistically significant. We further considered a more conservative Bonferroni corrected P value of <0.01 based on the number of performed tests (0.05/5= 0.01).

Results

Participants and characteristics

In total, 2,178 participants of the Rotterdam Study were included in this analysis.

Mean age was 68.6 ± 6.6 years, and 55% of the participants were women (Table 1). The ascending aortic (AA) diameter could be measured in 99.8% (2174/2178) and the descending aortic (DA) diameter in 98.5% (2146/2178) of the participants. Significant differences were observed in anthropometric measures, with height, weight, waist circumference and WHR being larger in men and BMI and hip circumference being higher in women. Men smoked more often, had a higher alcohol consumption as well as larger values for diastolic blood pressure, and CAC. Serum levels of total and HDL cholesterol were higher in women (Table 1).

During 13 years of follow-up (median: 10.6 years; range between 0.18-13.6 years), 64 women and 64 men were diagnosed with stroke, 53 women and 88 men developed CHD, and 72 women and 68 men developed HF. In total, 229 women and 255 men died during follow-up of which 46 and 39 deaths were due to cardiovascular causes among women and men respectively.

Crude and indexed ascending aortic diameters in association with cardiovascular outcomes and mortality

When analyzed continuously, larger crude AA diameter showed a significant association with stroke in only men (HR per 1 standard deviation (SD) increase; 95% CI: 1.35; 1.05, 1.75). Larger crude and height-indexed AA diameters showed a significant association with incident of HF among women (HR per 1 SD; 95% CI: 1.32; 1.03, 1.71 for crude and 1.37; 1.09, 1.71 for height-indexed diameter). Weight-indexed (HR per 1 SD; 95% CI: 1.35; 1.03, 1.77) and BSA-indexed (HR per 1 SD; 95% CI: 1.34; 1.005, 1.79) AA diameters showed a significant association with cardiovascular mortality in only women (Table 2, figure 1 A). Weight-indexed and BMI-indexed AA diameters showed a significant association with all-cause mortality among both women (HR per 1 SD; 95% CI: 1.19; 1.05, 1.36 for weight-indexed and 1.20; 1.06, 1.36 for BMI-indexed) and men (HR per 1 SD; 95% CI: 1.38; 1.16, 1.65 for weight-indexed and 1.31; 1.13, 1.53 for BMI-indexed).

Crude and indexed descending aortic diameters in association with cardiovascular outcomes and mortality

There was a stronger association between larger crude and indexed DA diameters with the risk of stroke, HF, cardiovascular mortality and all-cause mortality in women than men. The HRs per 1 SD (95% CI) for crude DA diameter were 1.38 (1.03, 1.84) for stroke, 1.33 (1.01, 1.74) for HF, 1.47 (1.05, 2.05) for cardiovascular

mortality, and 1.20 (1.02, 1.40) for all-cause mortality in women. Among men, only weight-indexed (HR per 1 SD; 95% CI: 1.47; 1.21, 1.77), BSA-indexed (HR per 1 SD; 95% CI: 1.19; 1.02, 1.38) and BMI-indexed (HR per 1 SD; 95% CI: 1.38; 1.17, 1.62) DA diameters were associated with all-cause mortality (Table 3, Figure 1 B).

Competing risk analyses for cardiovascular and all-cause mortality

Findings from the competing risk analyses showed significant associations for crude and all indexed DA diameters with cardiovascular mortality in women and for weight-indexed and BMI-indexed DA diameters with non-cardiovascular mortality in men. Neither crude nor indexed AA diameter showed any significant associations with cardiovascular or non-cardiovascular mortality among women or men in these analyses. (Supplemental eTable 1).

Associations of aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles

Supplemental eTables 2-5 show the cutoffs for crude values significantly associated with each outcome using the percentiles approach. In general, the area under the curve (AUC) for the examined percentiles were good (0.74-0.86) (eTables 6-9). For the ascending aorta, a crude diameter ≥ 41 mm (95th percentile) was associated with increased risk for CHD (HR; 95% CI: 2.76; 1.077, 7.09) and HF (HR; 95% CI: 3.25; 1.43, 7.38) among women and a crude AA diameter ≥ 40 mm (75th percentile) was associated with increased risk for stroke (HR; 95% CI: 2.05; 1.20, 3.50) among men as well as the cutoffs based on the 80th, 85th and 90th percentile. For descending aorta in women, crude diameter ≥ 31 mm (95th percentile) was associated with stroke (HR; 95% CI: 2.0; 1.03, 3.90) and a crude diameter ≥ 29 mm (75th percentile) was associated with HF (HR; 95% CI: 1.92; 1.16, 3.18) together with the cutoffs based on the 80th, 85th and 90th percentiles. A crude diameter ≥ 30 mm (85th percentile) was associated with cardiovascular mortality (HR; 95% CI: 2.37; 1.23, 4.55) as well as the cutoffs based on the 90th and 95th percentile. In men, crude DA diameter ≥ 31 mm (75th percentile) was associated with stroke (HR; 95% CI: 1.89; 1.11, 3.23).

Discussion

Within the large population-based Rotterdam Study, we found gender differences in the association of thoracic aortic diameters with the risk of cardiovascular outcomes during 13 years of follow-up. The gender differences were more pronounced for descending aorta, as the descending aortic diameters were strongly associated with stroke, HF, cardiovascular mortality in women. The risk for several cardiovascular outcomes increased significantly at cutoff points that were below the 95th percentile of the distribution of aortic diameters.

So far, few studies have shown associations between aortic root diameters and adverse cardiovascular outcomes[5,13,14], and the evidence regarding ascending and descending aortic diameters remains limited. Gondrie et al [6] showed that ascending and descending thoracic aortic diameters were associated with a borderline increased risk for CVD. Recently, the investigators from the Framingham Heart Study reported on the predictive value of enlarged descending aortic diameters for incident cardiovascular events in a younger population [7]. Both papers, however, were based on a composite cardiovascular outcome and did not evaluate associations in a gender specific manner. To our knowledge, our study is the first to determine the associations between ascending and descending aortic diameters with multiple cardiovascular outcomes among women and men separately.

In the current study, we report a stronger association between descending aortic diameter with cardiovascular outcomes and mortality than that of the ascending aortic diameter. This is in line with the literature regarding closer link between descending aortic aneurysms and atherosclerosis[15]. This relationship might be attributed to smaller diameters in descending aorta compared to the ascending aorta as well as to the possible different connective tissue content in the descending compared to the ascending thoracic aorta[16]. In a meta-analysis including 11 studies, Katsanos et al[15] showed a larger prevalence of complex atheromatous plaques in the descending aorta among patients with stroke compared with controls. Wehrum et al[17] showed that retrograde blood flow in the proximal region of descending aorta was found in patients with stroke and this retrograde blood flow increased in patients with concomitant complex plaques, low strain, and/or large aortic diameter. A larger prevalence of atheromatous plaques and a retrograde flow might explain the increased risk of stroke in participants with larger descending aortic diameters.

Sex-differences in atherosclerosis have been mainly found in the coronary vessels[18]. Consequently, the differences between women and men in prevalence of CVD and its risk markers have received considerable attention in patients with coronary

heart disease[19-21]. Our data revealed stronger associations between thoracic aortic diameters, in particular descending aorta, with the cardiovascular outcomes and mortality among women compared to men. As our population comprised post-menopausal women, hormonal changes during and after menopause may partly explain these findings. As women age and in particular following menopause, the prevalence of hypertension increases[22], which might partly explain the larger burden of stroke and HF in older women. However, the descending aortic diameter was independently associated with stroke, HF and cardiovascular mortality after taking into account hypertension among women in our study. Histopathological research of the descending aortic wall structure might help to further examine the differences between women and men with regard to the outcomes.

While both crude and indexed descending aortic diameters were associated with mortality, the associations for ascending aorta were stronger for indexed values. This suggests that it is important to adjust the ascending aortic diameters for body measurements to adequately predict cardiovascular outcomes. The importance of indexed values was also illustrated by Cuspidi et al[14], who showed that the aortic root indexed by height, but not by BSA or crude diameters, were associated with the composite outcome of fatal or non-fatal cardiovascular events.

The most recent European[23] and American[24] guidelines on primary prevention of CVD do not discuss the cardiovascular risk associated with increased thoracic aortic diameters. The European Society of Cardiology (ESC) guidelines consider patients with abdominal aortic aneurysms, but not those with thoracic aortic aneurysms, to be at very high cardiovascular risk[23]. We showed that the thoracic aortic diameter is associated with an increased risk of cardiovascular outcomes and mortality. In addition, we demonstrated that the risk for several cardiovascular outcomes significantly increases at a cutoff point below the sex-specific 95th percentiles of the population distribution. This implies that not only patients with aortic dilatation (aortic diameter >95th percentile) are at risk for major adverse outcomes, but a large group of individuals with mildly enlarged aortic diameters in the general population are at increased risk for cardiovascular outcomes and mortality.

Strengths of our study include the population-based setting and the relatively large sample size which made the sex-specific analyses with multiple outcomes possible. The use of ECG-gated CT scans allowed for highly accurate measurements of the aorta. Computed tomography is a commonly used imaging technique in clinical practice, which allows to measure the aortic diameter even when this is not the primary reason for the investigation. Furthermore, our analyses included an asymptomatic population free of prevalent CVD at baseline. Besides presenting

the associations with continuous aortic diameters, we also showed the predictive value of several clinically relevant percentile cut points to identify individuals at increased risk for cardiovascular events. The limitations of our study also merit acknowledgment. The Rotterdam study consists of participants of 55 years or older and predominantly from an European ethnicity. This might limit generalization of our results to younger and non-European populations. The performance of non-contrasted CT scan limits the visualization and differentiation of the components of the non-calcified atheromatous plaque at the level of the vascular wall. This does not allow us to evaluate the role that atherosclerosis at the level of the descending aorta can play in increasing the risk of stroke.

Conclusion

Our study suggests a role for the descending thoracic aortic diameter as a marker for increased cardiovascular risk, in particular among women. The cutoff point for increased cardiovascular risk for several of the cardiovascular outcomes, was below the 95th percentile of the distribution of thoracic aortic diameters.

Acknowledgements

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management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Competing Interests: None declared.

Contributors Planning: OLR-O, LRB, JWR-H, DB, MK, MWV, OHF, AVDL Conduct: OLR-O, LRB, JWR-H, DB, MK, RPJB, SR, KEG Reporting: OLR-O, LRB, JWR-H, DB, MK, RPJB, MWV, AVDL, SR, KEG, MKI, JWD, OHF.

Patient consent for publication: Obtained.

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Figure legends

Figure 1. Forest plot of hazard ratios for the association of crude ascending (A) and descending (B) aortic diameters with cardiovascular outcomes and mortality among 992 men and 1186 women
CHD= coronary heart disease, CV= cardiovascular.

Figure 2 Kaplan Meier curves for descending thoracic aortic diameter and cardiovascular outcomes in women using the crude sex-specific percentile derived cutoff values specified in each graph.
(A) stroke, (B) heart failure, (C) cardiovascular mortality

Table text

Table 1 Baseline characteristics of the participants

Values are presented as mean (standard deviation) or median (25^{th}-75^{th} percentile) or numbers (%).

Table 2. Standardized hazard ratios for crude and indexed ascending aortic diameters and risk of cardiovascular outcomes and mortality

† **Bold type:** Statistically significant association (P< 0.05). All models were adjusted for age, waist-hip ratio, smoking, alcohol consume, total and high-density lipoprotein cholesterol, hemoglobin, hypertension, diabetes mellitus, cardiovascular disease, lipid lowering, antithrombotic and blood pressure lowering medications and coronary and aortic arch calcifications. Weight and height were used as covariates for adjustment if they were not integrated into the indexed outcome. CV = cardiovascular, BSA = body Surface area, BMI = body mass index.

Table 3. Standardized hazard ratios for crude and indexed descending aortic diameters and risk of cardiovascular outcomes and mortality

† **Bold type:** Statistical significance association (P< 0.05). All models were adjusted for age, waist-hip ratio, smoking, alcohol consume, total and high-density lipoprotein cholesterol, hemoglobin, hypertension, diabetes mellitus, cardiovascular disease, lipid lowering, antithrombotic and blood pressure lowering medications and coronary and aortic arch calcifications. Weight and height were used as covariates for adjustment if they were not integrated into the indexed outcome. CV= cardiovascular, BSA=body Surface area, BMI: body mass index.

Table 1 Baseline characteristics of the participants

Variable	Total N= 2178	Women N=1186	Men N= 992	p-value
Age, years	68.6 (6.6)	68.7 (6.7)	68.6 (6.4)	0.7493
Weight, Kg	77.92 (13.58)	73.0 (12.7)	84 (12.1)	<0.001
Height, cm	167.9 (9.6)	161.6 (6.3)	175.3 (7.2)	<0.001
Body mass index	27.61 (4.16)	27.87 (4.58)	27.30 (3.56)	<0.002
Waist, cm	93.66 (11.6)	89.38 (11.0)	98.78 (10.1)	<0.001
Hip, cm	103.5 (7.8)	104.8 (8.9)	102.0 (5.9)	<0.001
Waist hip ratio	0.91 (0.09)	0.85 (0.07)	0.97 (0.07)	< 0.001
Systolic blood pressure, mmHg	147 ± 19.9	147.0 ± 20.3	146.4 ± 19.5	0.504
Diastolic blood pressure, mmHg	80.6 ± 10.6	79.5 ± 10.5	81.9 ± 10.6	<0.001
Smoking, n (%)				<0.001
Never	661 (30.4%)	494 (42%)	167 (17%)	
Past	1145 (52.6%)	512 (43%)	633 (64%)	
Current	372 (17%)	180 (15%)	192 (19%)	
Alcohol consumption, n (%)				<0.001
Never	136 (6.2%)	114 (10%)	22 (2%)	
Past	155 (7.1%)	89 (7%)	66 (7%)	
Current	1887 (86.7%)	983 (83%)	904 (91%)	
Coronary artery calcium (log+1)	3.35 (2.40)	2.7 (2.30)	4.1 (2.29)	<0.001
Aortic arch calcium (log+1)	4.9 (2.32)	4.8 (2.33)	5.0 (2.30)	0.164
Total cholesterol mmol/lt	5.8 ± 0.9	6.0 ± 0.9	5.5 ± 0.9	<0.001
HDL cholesterol mmol/lt	1.5 ± 0.4	1.6 ± 0.4	1.3 ± 0.3	<0.001

Variable	Total N= 2178	Women N=1186	Men N= 992	p-value
Hemoglobin gr/dl	8.9 ± 0.7	8.6 ± 0.6	9.3 ± 0.7	<0.001
Glucose mmol/lt	5.7 ± 1.2	5.6 ± 1.2	5.8 ± 1.2	0.010
Comorbidities, n (%)				
Prevalent hypertension	1318 (60.5%)	714 (60%)	604 (61%)	0.743
Prevalent diabetes mellitus	269 (12.4%)	140 (12%)	129 (13%)	0.482
Medication use, n (%)				
Blood pressure lowering medication	792 (36.4%)	439 (37%)	353 (36%)	0.486
Lipid lowering medication	421 (19.3%)	250 (21%)	171 (17%)	0.024
Antithrombotic agents	340 (15.6%)	161 (14%)	179 (18%)	0.004

Values are presented as mean (standard deviation) or median (25^{th}-75^{th} percentile) or numbers (%).

Table 2. Standardized hazard ratios for crude and indexed ascending aortic diameters and risk of cardiovascular outcomes and mortality

	Stroke	Coronary Heart Disease	Heart Failure	CV Mortality	Total Mortality
Women					
Crude diameter	1.06 (0.80, 1.41)	1.20 (0.89, 1.61)	**1.32 (1.03, 1.71)†**	1.19 (0.85, 1.65)	1.10 (0.95, 1.27)
Height Indexed	1.06 (0.82, 1.38)	1.17 (0.90, 1.54)	**1.37 (1.09, 1.71)†**	1.18 (0.87, 1.60)	1.06 (0.93, 1.22)
Weight Indexed	1.24 (0.96, 1.59)	1.15 (0.87, 1.51)	1.01 (0.80, 1.29)	**1.35 (1.03, 1.77)†**	**1.19 (1.05, 1.36) †**
BSA Indexed	1.22 (0.95, 1.57)	1.18 (0.90, 1.54)	1.24 (0.98, 1.56)	**1.34 (1.005, 1.79) †**	1.13 (0.99, 1.29)
BMI Indexed	1.19 (0.92, 1.53)	1.15 (0.87, 1.51)	0.96 (0.75, 1.22)	1.29 (0.99, 1.69)	**1.20 (1.06, 1.36) †**
Men					
Crude diameter	**1.35 (1.05, 1.75)†**	0.90 (0.71, 1.14)	0.95 (0.76, 1.24)	1.25 (0.92, 1.70)	1.05 (0.92, 1.20)
Height Indexed	1.29 (0.99, 1.66)	0.91 (0.72, 1.15)	0.97 (0.79, 1.50)	1.22 (0.9, 1.66)	1.0 (0.88,1.14)
Weight Indexed	1.41 (0.95, 2.10)	0.91 (0.66, 1.27)	0.95 (0.66, 1.38)	1.42 (0.92, 2.17)	**1.38 (1.16, 1.65) †**
BSA Indexed	1.25 (0.93, 1.68)	0.93 (0.71, 1.21)	0.99 (0.75, 1.30)	1.37 (0.97, 1.94)	1.12 (0.97, 1.29)
BMI Indexed	1.37 (0.98, 1.91)	0.91 (0.69, 1.21)	0.93 (0.68, 1.28)	1.32 (0.90, 1.93)	**1.31 (1.13, 1.53) †**

Table 3. Standardized hazard ratios for crude and indexed descending aortic diameters and risk of cardiovascular outcomes and mortality

	Stroke	Coronary Heart Disease	Heart Failure	CV Mortality	Total Mortality
Women					
Crude diameter	**1.38 (1.03, 1.84)** †	0.84 (0.60, 1.18)	**1.33 (1.01, 1.74)**†	**1.47 (1.05, 2.05)** †	**1.20 (1.02, 1.40)** †
Height Indexed	**1.33 (1.04, 1.72)** †	0.87 (0.65, 1.16)	**1.38 (1.10, 1.73)**†	**1.39 (1.05, 1.85)** †	1.12 (0.98, 1.28)
Weight Indexed	**1.40 (1.10, 1.79)** †	0.94 (0.70, 1.25)	0.99 (0.77, 1.27)	**1.48 (1.13, 1.95)** †	**1.22 (1.07, 1.39)** †
BSA Indexed	**1.48 (1.16, 1.89)** †	0.90 (0.67, 1.19)	1.24 (0.99, 1.56)	**1.52 (1.16, 1.99)** †	**1.17 (1.03, 1.34)** †
BMI Indexed	**1.37 (1.06, 1.76)** †	0.93 (0.70, 1.25)	0.94 (0.73, 1.22)	**1.46 (1.09, 1.95)** †	**1.23 (1.08, 1.41)** †
Men					
Crude diameter	1.32 (0.97, 1.79)	1.08 (0.83, 1.41)	1.16 (0.88, 1.54)	1.02 (0.66, 1.56)	1.12 (0.96, 1.31)
Height Indexed	1.26 (0.95, 1.67)	1.08 (0.84, 1.38)	1.16 (0.89, 1.51)	1.00 (0.68, 1.49)	1.05 (0.91, 1.20)
Weight Indexed	1.21 (0.80, 1.83)	1.11 (0.80, 1.55)	1.08 (0.76, 1.55)	1.26 (0.76, 2.09)	**1.47 (1.21, 1.77)** †
BSA Indexed	1.14 (0.83, 1.57)	1.12 (0.86, 1.46)	1.16 (0.88, 1.54)	1.21 (0.81, 1.81)	**1.19 (1.02, 1.38)** †
BMI Indexed	1.21 (0.85, 1.73)	1.07 (0.80, 1.43)	1.04 (0.76, 1.43)	1.17 (0.75, 1.83)	**1.38 (1.17, 1.62)** †

Figure 1

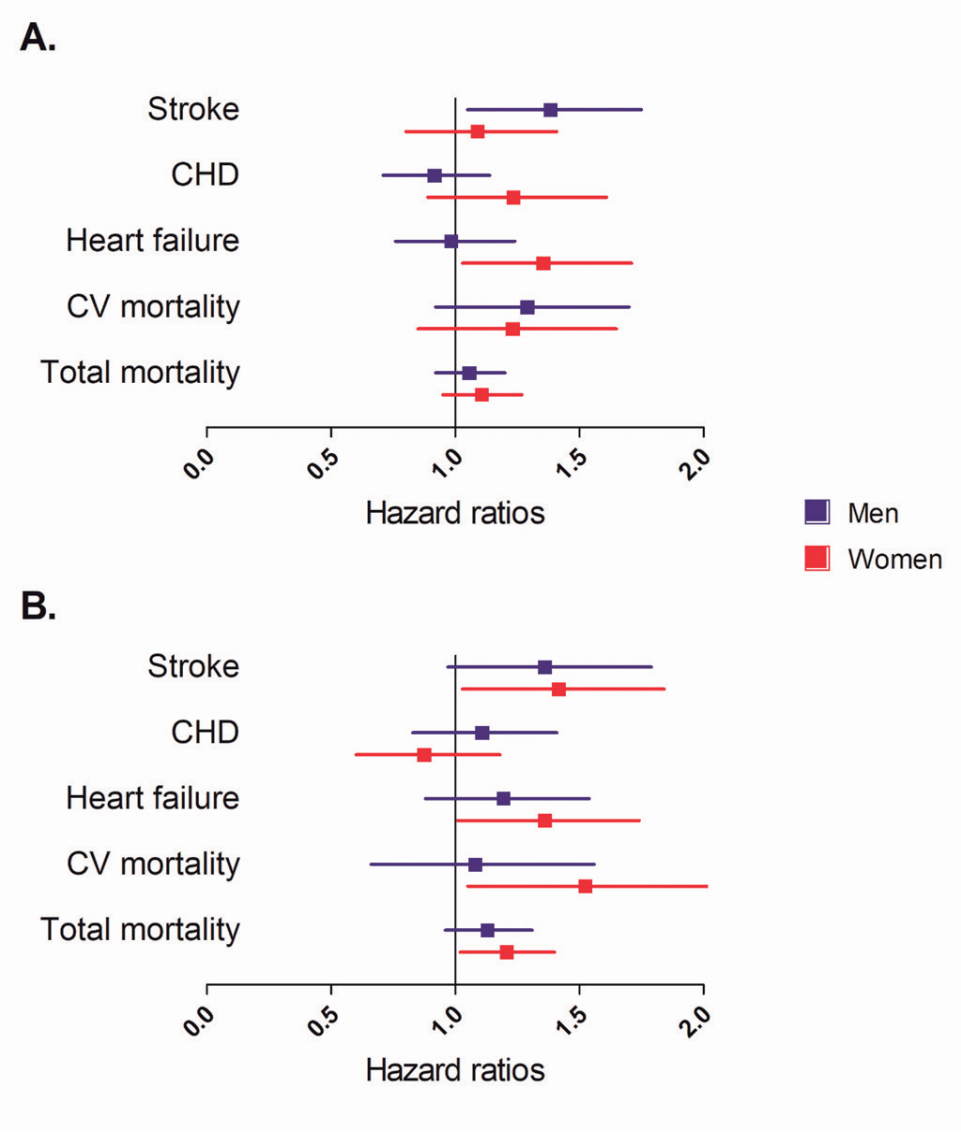

A.
Stroke
CHD
Heart failure
CV mortality
Total mortality
0.0
0.5
1.0
1.5
2.0
Hazard ratios
Men
Women
B.
Stroke
CHD
Heart failure
CV mortality
Total mortality
0.0
0.5
1.0
1.5
2.0
Hazard ratios

Figure 2A

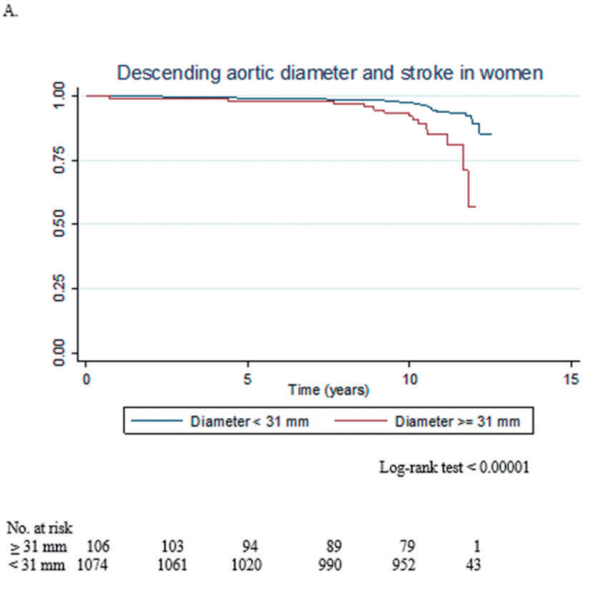


Figure 2B

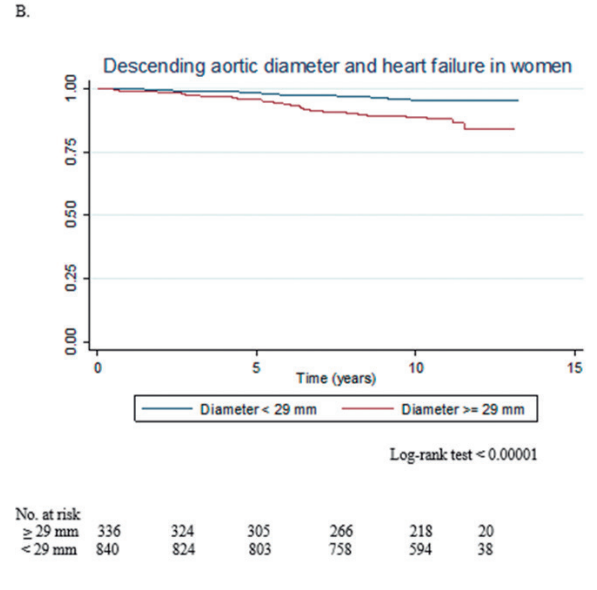
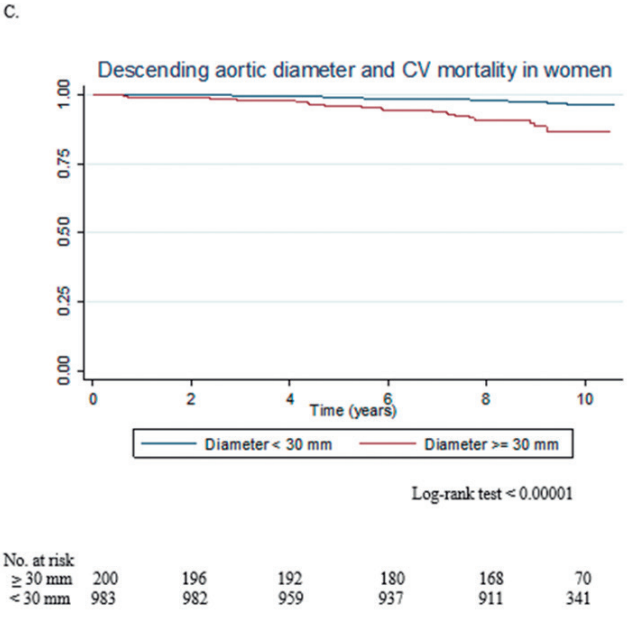


Figure 2C



No. at risk						
≥ 30 mm	200	196	192	180	168	70
< 30 mm	983	982	959	937	911	341

SUPPLEMENTAL MATERIAL

Rueda-Ochoa OL, Bons LR, Rohde S, et al. Thoracic aortic diameter and cardiovascular events and mortality among women and men.

eAppendix 1. Assessment of determinants

eAppendix 2 Cardiovascular and mortality outcomes

eTable 1. Baseline characteristics for participants with aortic dissection during follow-up

eTable 2. Competing sub- hazard ratios for crude and indexed thoracic aortic diameters and risk of cardiovascular and non-cardiovascular mortality.

eTable 3. Associations of crude ascending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among women

eTable 4. Associations of crude ascending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among men

eTable 5. Associations of crude descending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among women

eTable 6. Associations of crude descending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among men

eTable 7. Area under the curve for crude ascending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in women.

eTable 8. Area under the curve for crude ascending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in men.

eTable 9. Area under the curve for crude descending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in women

eTable 10. Area under the curve for crude descending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in men.

eReferences

eAppendix 1. Assessment of determinants

Data on current and former smoking habits and alcohol consumption were collected during a structured home interview. We assessed information on the use of blood pressure lowering medication, antithrombotic agents and lipid modifying agents including statins according to the ATC/DDD system (http://www.whocc.no). Clinical measurements were collected during regular visits at the research center. Body surface area (BSA) was calculated using the Dubois formula:[1] BSA (in m^2) = 0.007184 x height (in m)$^{0.725}$ x weight (in kg)$^{0.425}$. In addition, waist and hip circumference were assessed. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position. The average of three consecutive measurements was used. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg and/or the use of blood pressure-lowering medication. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured using an automated procedure. Diabetes was defined as the use of glucose-lowering medication and/or a fasting glucose level ≥7 mmol/L. Coronary artery calcification (CAC) and aortic arch calcification (AAC) volumes were quantified using commercially available software (Syngo CalciumScoring; Siemens).

eAppendix 2. Cardiovascular and mortality outcomes

The major adverse outcomes included in this study were stroke, coronary heart disease (CHD), heart failure (HF), cardiovascular mortality and all-cause mortality. All Rotterdam Study participants are intensively and constantly followed to identify the development of all adverse outcomes.

Stroke was defined according to World Health Organization criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 h or longer or leading to death, with no apparent origin other than vascular. History of stroke, cardiovascular disease (CVD) or HF at baseline was assessed during the baseline interview and verified by reviewing

medical records. After enrollment, participants were continuously monitored for incident of stroke, CVD or HF through automated linkage of the study database with medical records from general practitioners. Additional information was obtained from hospital records. Potential strokes identified in medical records were reviewed by research physicians, and verified by an experienced stroke neurologist. Transient ischemic attacks or subarachnoid hemorrhages were not included. Follow-up was complete until January 1st, 2016[2]. Incidence of CHD included incidence of fatal or non-fatal myocardial infarction (MI), other CHD mortality, coronary artery bypass graft, or percutaneous transluminal angioplasty during follow-up until January 1st, 2015, confirmed by ECG or medical records[3]. The diagnosis of HF was classified as definite, probable, possible or unlikely. Definite HF was defined as a combination of the presence of typical symptoms or signs of HF, such as breathlessness at rest or during exertion, ankle edema, and pulmonary crepitation, confirmed by objective evidence of cardiac dysfunction (chest X-ray, echocardiography)[3]. This definition is in accordance with the criteria of the European Society of Cardiology (ESC)[4]. HF is classified as probable when at least two typical symptoms suggestive of HF are present, and at least one of the following: history of CVD (e.g., MI, valvar heart disease, hypertension), positive response to initiated treatment for HF, or objective evidence of cardiac dysfunction, while symptoms cannot be attributed to another underlying disease, such as chronic obstructive pulmonary disease. In accordance with the ESC guidelines, only definite and probable cases are used in the Rotterdam Study definition. HF follow-up information was available until January 1st, 2015. Information on the vital status of all participants was obtained on April 20, 2017 from the central registry of the municipality in Rotterdam and through digital linkage with records from general practitioners working in the study area. Cardiovascular mortality was available until January 1st, 2015. The cause of death was established by abstracting information from the medical records of the general practitioners or nursing home physicians and hospital discharge letters. Cardiovascular mortality was defined according to the World Health Organization including ICD-10 codes I00-I99, as described in detail elsewhere[5].

	Ascending aorta				Descending aorta			
	Cardiovascular mortality		Non-cardiovascular mortality		Cardiovascular mortality		Non-cardiovascular mortality	
	Sub-Hazard	95% CI	Sub-Hazard	95% CI	Sub-Hazard	95% CI	Sub-Hazard	95% CI
Women								
Crude diameter	0.99	0.72, 1.36	1.09	0.88, 1.35	**1.58**	**1.10, 2.26 †**	0.97	0.78, 1.20
Height Indexed	1.00	0.76, 1.33	1.07	0.88, 1.31	**1.48**	**1.10, 1.99 †**	0.95	0.79, 1.15
Weight Indexed	1.14	0.84, 1.55	1.12	0.91, 1.38	**1.38**	**1.04, 1.84 †**	1.03	0.85, 1.25
BSA Indexed	1.10	0.81, 1.49	1.08	0.88, 1.34	**1.50**	**1.12, 2.00 †**	0.97	0.80, 1.17
BMI Indexed	1.11	0.82, 1.50	1.14	0.93, 1.39	**1.36**	**1.02, 1.82 †**	1.06	0.87, 1.29
Men								
Crude diameter	1.06	0.85, 1.32	0.92	0.78, 1.09	0.86	0.65, 1.14	1.05	0.87, 1.26
Height Indexed	1.07	0.88, 1.30	0.87	0.74, 1.03	0.89	0.69, 1.16	0.97	0.81, 1.15
Weight Indexed	1.13	0.83, 1.53	1.20	0.96, 1.50	0.99	0.71, 1.40	**1.37**	**1.08, 1.74 †**
BSA Indexed	1.15	0.93, 1.41	0.95	0.79, 1.15	0.99	0.75, 1.30	1.07	0.89, 1.28
BMI Indexed	1.13	0.85, 1.50	1.18	0.97, 1.43	0.99	0.72, 1.34	**1.33**	**1.08, 1.64 †**

eTable 1. Competing sub- hazard ratios for crude and indexed thoracic aortic diameters and risk of cardiovascular and non-cardiovascular mortality.

† **Bold type:** Statistically significant association (P< 0.05). BSA = Body Surface area, BMI = Body mass index.

All models were adjusted for age, waist-hip ratio, smoking, alcohol consume, total and HDL cholesterol, hemoglobin, hypertension, diabetes mellitus, cardiovascular disease, lipid lowering antithrombotic and anti-hypertensions medications and coronary and aortic arch calcifications. Weight and height were used as covariates for adjustment if they were not integrated into the indexed outcome.

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	37 mm	1.60 (0.92, 2.76)	1.28 (0.71, 2.30)	1.20 (0.70, 2.10)	1.53 (0.80, 2.91)	1.16 (0.86, 1.55)
P80	38 mm	1.76 (0.97, 3.19)	1.22 (0.63, 2.36)	1.71 (0.98, 2.99)	1.64 (0.81, 3.31)	1.17 (0.84, 1.63)
P85	39 mm	1.76 (0.97, 3.19)	1.22 (0.63, 2.36)	1.71 (0.98, 2.99)	1.64 (0.81, 3.31)	1.17 (0.84, 1.63)
P90	40 mm	1.028 (0.46, 2.29)	1.29 (0.59, 2.79)	1.36 (0.70, 2.64)	0.81 (0.28, 2.29)	1.10 (0.75, 1.62)
P95	41 mm	-	**2.76 (1.077, 7.09)**	**3.25 (1.43, 7.38)**	0.53 (0.072, 3.88)	1.21 (0.67, 2.19)

eTable 2. Associations of crude ascending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among women
Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	40 mm	**2.05 (1.20, 3.50)**	0.91 (0.55, 1.51)	1.06 (0.61, 1.85)	1.76 (0.87, 3.55)	1.22 (0.91, 1.62)
P80	40 mm	**2.05 (1.20, 3.50)**	0.91 (0.55, 1.51)	1.06 (0.61, 1.85)	1.76 (0.87, 3.55)	1.22 (0.91, 1.62)
P85	41 mm	**1.93 (1.02, 3,67)**	0.48 (0.22, 1.07)	0.52 (0.22, 1.26)	1.31 (0.52, 3.30)	1.20 (0.83, 1.73)
P90	42 mm	**1.93 (1.02, 3.67)**	0.48 (0.22, 1.07)	0.52 (0.22, 1.26)	1.31 (0.52, 3.30)	1.20 (0.83, 1.73)
P95	43 mm	1.99 (0.98, 4.04)	0.40 (0.14, 1.11)	0.65 (0.25, 1.72)	1.25 (0.42, 3.74)	1.13 (0.73, 1.76)

eTable 3. Associations of crude ascending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among men
Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	29 mm	1.21 (0.70, 2.10)	0.59 (0.31, 1.13)	**1.92 (1.16, 3.18)**	1.50 (0.79, 2.82)	1.13 (0.85, 1.51)
P80	29 mm	1.21 (0.70, 2.10)	0.59 (0.31, 1.13)	**1.92 (1.16, 3.18)**	1.50 (0.79, 2.82)	1.13 (0.85, 1.51)
P85	30 mm	1.71 (0.94, 3.11)	0.95 (0.48, 1.89)	**1.85 (1.08, 3.17)**	**2.37 (1.23, 4.55)**	1.36 (0.99, 1.85)
P90	30 mm	1.71 (0.94, 3.11)	0.95 (0.48, 1.89)	**1.85 (1.08, 3.17)**	**2.37 (1.23, 4.55)**	1.36 (0.99, 1.85)
P95	31 mm	**2.0 (1.03, 3.90)**	0.84 (0.36, 1.95)	1.46 (0.76, 2.82)	**2.62 (1.28, 5.36)**	1.38 (0.96, 1.98)

eTable 4. Associations of crude descending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among women
Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	31 mm	**1.89 (1.11, 3.23)**	0.99 (0.62, 1.58)	1.37 (0.80, 2.33)	0.93 (0.45, 1.92)	1.13 (0.88, 1.50)
P80	32 mm	1.15 (0.66, 2.03)	1.24 (0.76, 2.04)	1.31 (0.76, 2.25)	1.06 (0.50, 2.25)	1.21 (0.91, 1.60)
P85	32 mm	1.15 (0.66, 2.03)	1.24 (0.76, 2.04)	1.31 (0.76, 2.25)	1.06 (0.50, 2.25)	1.21 (0.91, 1.60)
P90	33 mm	1.39 (0.73, 2,68)	0.98 (0.51, 1.88)	1.32 (0.69, 2.52)	0.93 (0.36, 2.38)	1.22 (0.87, 1.72)
P95	34 mm	0.74 (0.28, 1.95)	1.07 (0.47, 2.43)	1.0 (0.40, 2.49)	103 (0.29, 3.63)	1.09 (0.69, 1.74)

eTable 5. Associations of crude descending thoracic aortic diameters with cardiovascular outcomes at dichotomous cut points corresponding to 75th, 80th, 85th, 90th, and 95th percentiles among men
Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	37 mm	0.7748	0.8044	0.7753	0.8508	0.7573
P80	38 mm	0.7788	0.8035	0.7820	0.8506	0.7569
P85	39 mm	0.7788	0.8035	0.7820	0.8506	0.7569
P90	40 mm	0.7663	0.8035	0.7771	0.8485	0.7568
P95	41 mm	-	0.8078	0.7858	0.8478	0.7561

eTable 6. Area under the curve for crude ascending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in women
Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	40 mm	0.7755	0.7595	0.8034	0.8618	0.7421
P80	40 mm	0.7755	0.7595	0.8034	0.8618	0.7421
P85	41 mm	0.7631	0.7664	0.8081	0.8535	0.7409
P90	42 mm	0.7631	0.7664	0.8081	0.8535	0.7409
P95	43 mm	0.7662	0.7694	0.8070	0.8538	0.7408

eTable 7. Area under the curve for crude ascending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in men
Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	29 mm	0.7663	0.8057	0.7876	0.8537	0.7577
P80	29 mm	0.7663	0.8057	0.7876	0.8537	0.7577
P85	30 mm	0.7702	0.8035	0.7860	0.8597	0.7603
P90	30 mm	0.7702	0.8035	0.7860	0.8597	0.7603
P95	31 mm	0.7752	0.8032	0.7816	0.8682	0.7605

eTable 8. Area under the curve for crude descending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in women
Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

Diameter		Stroke	Coronary heart disease	Heart failure	Cardiovascular mortality	Total mortality
P75	31 mm	0.7700	0.7554	0.8102	0.8573	0.7412
P80	32 mm	0.7595	0.7571	0.8114	0.8587	0.7407
P85	32 mm	0.7595	0.7571	0.8114	0.8587	0.7407
P90	33 mm	0.7615	0.7554	0.8090	0.8580	0.7403
P95	34 mm	0.7597	0.7553	0.8075	0.8579	0.7401

eTable 9. Area under the curve for crude descending thoracic aortic diameter cut points (corresponding to 75th, 80th, 85th, 90th, and 95th percentiles) in association with the risk of cardiovascular outcomes in men. Models include: age, weight, height, waist-hip ratio, hypertension, Smoking, alcohol, Prevalence of diabetes mellitus, lipid lower medications, anti-thrombotic medications, total cholesterol, hdl cholesterol, hemoglobin, cohort, type of scanner, and coronary and aortic arch calcifications. † **Bold type:** Statistically significant association (P< 0.05).

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CHAPTER 4.3

Association of coronary artery disease genetic risk score with atherosclerosis in various vascular domains

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ABSTRACT

Background

Genomic-wide association studies (GWAS) have identified multiple risk loci for the development of coronary artery disease (CAD). However, the involvement of these genetic variants in atherosclerosis in various vascular beds and wether they differ remain unknown. We constructed a genetic risk score (GRS), based on the identified 160 CAD genetic variants to examine the extent to which the genetic predisposition for CAD would explain the variation in clinical and subclinical atherosclerosis in different vascular domains. Moreover, we assessed the association of eight specific GRS for CAD, representing different biological pathways, with all phenotypes under study.

Methods

We calculated a weighted GRS using 160 independent single nucleotide polymorphisms (SNPs) associated with CAD. Associations of the GRS with subclinical measures of atherosclerosis including common carotid intima media thickness (cIMT) and carotid plaque, ankle brachial index (ABI), pulse wave velocity (PWV), ascending thoracic aorta diameter, descending thoracic aorta diameter, abdominal aortic diameter (AAD), coronary artery calcification (CAC), aortic arch calcification (AAC), extra-cranial carotid artery calcification (ECAC) and intra-cranial carotid artery calcification (ICAC) were assessed using linear and logistic regression models. To investigate the association of the GRS with prevalent and incident coronary heart disease (CHD), stroke and incident CVD mortality, we used logistic regression and Cox proportional hazards models respectively.

Results

Global CAD GRS showed significant associations, per one unit of increased in Standard deviation, with carotid plaque Odds Ratio (OR):1.09, (95% Confidence

interval (CI) 1.05, 1.14), CAC Beta (β): 0.20, (0.088, 0.32), AAC β: 0.15 (0.045, 0.25), ECAC β: 0.14 (0.038, 0.23), ICAC β: 0.11 (0.013, 0.20), prevalent CHD OR:1.33 (1.23, 1.43) , incident CHD HR:1.14 (1.08, 1.20) and incident CVD mortality HR=1.11 (1.03, 1.20). There was no association between global CAD GRS with IMT, ABI, PWV, ascending thoracic and abdominal aortic diameters, prevalent and incident stroke, and prevalence of peripherical artery disease (PAD). Transcription, inflammation and remodeling were the biological pathways more frequently associated with phenotypes under study.

Conclusions

High genetic predisposition for CAD showed a differential association with clinical CVD events and subclinical measures of atherosclerosis in various vascular beds. These findings could indicate genetic heterogeneity in atherosclerosis process in different vascular vessels .

Keywords: Genetic risk score, Coronary artery disease, Atherosclerosis, Vascular beds, Cohort study.

introduction

Atherosclerosis is a process of degeneration of the vascular intima, which begins in the intra-uterine life and progresses gradually, influenced by the genetic and environmental factors defined as cardiovascular risk factors [1]. Atherosclerosis is considered a progressive and systemic disease that affects different blood vessels. However, the correlation between different vascular measures of atherosclerosis varies[2].

Coronary artery disease (CAD) is among the most common clinical manifestations of atherosclerosis. Recently, Genomic-Wide Association Studies (GWAS) have identified multiple risk loci for the development of CAD [3-16]. However, the association of these genetic variants for atherosclerosis with various vascular beds is not well known. While atherosclerotic diseases in different vascular domains share common risk factors, increasing evidence suggests that genetic variants might play a distinct role in various vessel beds [2]. However, there is little information on different biological pathways underlying atherosclerosis in various vascular beds and clinical and subclinical phenotypes.

We aimed to develop a genetic risk score (GRS), based on all identified CAD genetic variants [10-16], and examine the extent to which this score would explain the variation in atherosclerosis in different vascular domains. In particular, we sought to assess the GRS for CAD in association with subclinical measures of atherosclerosis in different vessels including coronary, carotid, and peripheral arteries as well as different clinical manifestations of atherosclerosis including coronary heart disease (CHD), stroke, and peripheral arterial disease (PAD). We also investigated whether different biological pathways are involved in the associations with clinical and subclinical atherosclerotic phenotypes under study.

MATERIALS AND METHODS

Study population

This study was performed within the framework of the Rotterdam Study (RS), a

prospective, population-based cohort study which started in 1990 in the well-defined Ommoord district in the city of Rotterdam, the Netherlands. The original cohort of the Rotterdam Study (RS-I) comprised 7983 participants who were 55 years or older. Since

then, the cohort has been extended twice to include participants who reached the age of 55 years after the original cohort and persons aged 55 years or older who migrated into the research area. The second cohort (RS-II) included 3011 individuals, and the third cohort (RS-III) included 3932 Participants who were all interviewed at home followed by an extensive set of physical examinations in a specially built research facility. Examinations were repeated every 3-5 years. The current analysis was performed to 11496 participants (6672 women and 4824 men) from the first visit of the original cohort (RS-I-1, n:6291), the first visit of the second extended cohort (RS-II-1, n:2157) and the first visit of the third extended cohort (RS-III-1, n:3048) , among whom both genotype data and complete information of IMT, carotid plaque, ABI, PWV, PAD and incidence of CHD, stroke and cardiovascular death were available. The analysis of CAC, AAC, ECAC, ICAC and thoracic aortic diameters was made in 2057 (1045 women and 1012 men) participants from RS-I4 and RS-II2, with complete genetic and subclinical atherosclerosis information. The RS has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants in the present analysis provided written informed consent to participate and to obtain information from their treating physicians[17].

Measurements

Genotyping

All study participants were genotyped with the 550K, 550K duo, or 610 quad Illumina arrays. We removed samples with a call rate <97.5%, sex mismatch, excess autosomal heterozygosity (>0.336), duplicates or family-relations, and ethnic outliers. Moreover, we removed those variants with failing missingness tests, Hardy–Weinberg equilibrium P values $<10^{-6}$, and minor allele frequencies (MAF) of <0.1%. Genotypes were imputed using MACH/minimac software to the 1000 Genomes phase I version 3 reference panel (entire population).[18,19].

Genetic Risk Score

To construct the GRS, we searched PubMed using key words 'genome-wide association study', 'GWAS', 'coronary artery disease' and 'CAD' and identified a summary on the genetics of CAD in the last decade involving more than 350 000 people. For this study, we selected 160 uncorrelated single nucleotide polymorphisms (SNPs) associated with CAD that were available in the RS imputed dataset[16]. Supplementary table 1 provides an overview of the SNPs included in the genetic score for CAD. The effect allele (coded 0–2) was the CAD raising allele. We then calculated the GRS by multiplying the number of risk alleles at each locus by corresponding reported beta-coefficient and summed the products. The GRS was computed using RStudio (R Foundation for Statistical Computing, Vienna, Austria).

The SNPs included in the GRS were not in linkage desequilibrium (LD). LD was investigated as the correlation among the genetic variants, using the correlation parameter R squared (R^2). R^2 lower than < 0.3 was considered the threshold for the tested variants to not be in LD. R^2 was assessed using the web-based application LDlink (https://ldlink.nci.nih.gov).

Subclinical measures of atherosclerosis

In this study, we used the following outcomes as subclinical measures of atherosclerosis based on biological plausibility and current literature: common carotid intima media thickness (cIMT) and carotid plaque, ankle brachial index (ABI), pulse wave velocity (PWV), ascending and descending thoracic aorta diameter (ATAD, DTAD), abdominal aortic diameter (AAD), coronary artery

calcification (CAC), aortic arch calcification (AAC), extra-cranial carotid artery calcification (ECAC) and intra-cranial carotid artery calcification (ICAC).

Ultrasonography of the common carotid artery, carotid bifurcation and internal carotid artery in both sides was performed to measure cIMT using a 7.5 Mhz linear array transducer (ATL Ultramar IV). Two bright white lines separated by a hypo echogenic space were displayed in a longitudinal view. The distance between the leading edge of the first and second bright lines indicated the intima media thickness. The cIMT, summarized as the mean of the maximal measurements from the near and far walls on both the left and right sides, was used for these analyses. The common carotid artery, carotid bifurcation, and internal carotid artery were examined on both the left and right sides for the presence of plaques as previously described[20,21]. ABI is the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm. ABI was measured in both legs following the RS protocol and the lower value was used [22]. PAD was defined as ABI values lower than 0.9 and history of intermittent claudication. PWV was measured with an automatic device (Complior; Artech Medical, Pantin, France) which measures the time delay between the rapid upstroke of simultaneously recorded pulse waves in the carotid and the femoral arteries in meters per second [23]. Both ascending and descending aortic thoracic diameters were obtained by non-contrast CT images using 16-slice or 64-slice multidetector CT scanners (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany). Detailed information on the scan protocol has been provided previously.[24] AAD was measured using a commercially available ultrasonography system (AU3 Partner, Esaote Biomedica) with a 3.5/2.5 Mhz transducer. Both anteroposterior and transversal diameters were measured. CAC was measured in the epicardial coronary arteries with either a C-150 electron beam computed tomography scanner (Imatron, South San Francisco, California) or a 16- or 64-slice multidetector computed tomography scanner (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany) and quantified as Agatston score[24]. A semi automated scoring method was used for quantification of calcification in ICAC bilaterally using consecutive CT sections. Calcification volumes of the others vessels were quantified using an automated software (Syngo calcium scoring; Siemens)[25].

Clinical Cardiovascular Disease

Clinical cardiovascular (CVD) outcomes included both prevalent and incident CHD and stroke as well as incident CVD mortality. Incident CHD was defined as fatal or non-fatal myocardial infarction, coronary revascularization, or death from CHD as described previously [26]. Strokes were diagnosed when a patient had the typical

neurological symptoms. Diagnosis was confirmed using computed tomography or magnetic resonance imaging, made within 4 weeks after the occurrence of stroke.[27]. Follow-up data was collected through general practitioners in the study area and subsequent collection of information from letters of medical specialists and discharge reports in case of hospitalization. Follow up was completed until January 2016. CVD mortality was mainly attributed to myocardial infarction, ischemic heart failure or sudden death and it was classified using a marginally adapted classification applied by both Atherosclerotic Risk in Community Study and the Cardiovascular Health Study[26].

Statistical analysis

Participant characteristics were described as mean (standard deviation) for continuous variables and proportions for dichotomous variables. To assess the association of the GRS with different subclinical measures of atherosclerosis, linear and logistic regression analyses were performed. Continuous and dichotomous subclinical atherosclerosis measures were used as the dependent variable and the GRS as the independent variable. To assess the association of the GRS with prevalent and incident different cardiovascular outcomes, we used logistic regression and Cox proportional hazards models respectively. For each analysis, the main exposure was standardized weigthed CAD GRS for all 160 SNPs or eight standardized CAD GRS for specific biological pathways independently, that included lipid metabolism, blood pressure, inflammation, vascular remodeling, neovascularization/angiogenesis, nitric oxide signaling, mitosis proliferation and transcription gene regulation. For all models including subclinical measures of atherosclerosis as outcome, we computed the amount of explained variance by the GRS. A secondary analysis was made bassed on CAD GRS quartiles for all 160 SNPs and CAD GRS for each biological pathway under study. The analyses were performed with STATA V14.0. A 2-sided P value of less than 0.05 denoted statistical significance. We further considered a stringent Bonferroni adjusted P value of 0.0033 adjusted for the number of outcomes under study (0.05/15).

Results

Population Characteristics

General characteristics of the 11946 participants are presented in Table 1. Mean (standard deviation – SD) age of the population was 65.32 (9.91) years. Mean

(SD) BMI was 26.85 (4.07). 30.1% and 9.7% of the population were receiving anti-hypertensive and lipid lowering medications respectively. Prevalences of DM, CHD, and stroke were 11.93%, 14.05%, and 7.28% respectively. Family history of CVD was reported by 51.67% of study participants. Mean (SD) GRS was 11.04 (0.49) and the GRS range was between 9.18 to 12.92. Details regarding the 160 included SNPs in the GRS are presented in Supplemental Table 1.

Association of global CAD GRS with clinical and subclinical atherosclerosis

Global CAD GRS showed significant associations, per one unit of increased in standard deviation, with carotid plaque Odds Ratio (OR):1.09, (95% Confidence interval (CI) 1.05, 1.14), descending TAD Beta (β): -0.15 (-0.27, -0.04), CAC β: 0.20, (0.088, 0.32), AAC β: 0.15 (0.045, 0.25), ECAC β: 0.14 (0.038, 0.23), ICAC β: 0.11 (0.013, 0.20), prevalent CHD OR:1.33 (1.23, 1.43) , incident CHD HR:1.14 (1.08, 1.20) and incident CVD mortality HR=1.11 (1.03, 1.20). There was no association of global CAD GRS with IMT, ABI, PWV, ascending thoracic and abdominal aortic diameters, prevalent and incident stroke, and prevalent of peripherical artery disease (PAD) (Table 2).

Association of specific CAD GRS, based on involved biological pathways, with clinical and subclinical atherosclerosis

Eight specific CAD GRS of involved biological pathways were build from the 160 SNPs of Global CAD GRS. The biological pathways included: 1) lipid metabolism (17 SNPs), 2) blood pressure (7 SNPs), 3) inflammation (15 SNPs), 4) vascular remodeling (23 SNPs), 5) neovascularization/angiogenesis (9 SNPs), 6) nitric oxide signaling (7 SNPs), 7) mitosis proliferation (16 SNPs) and, 8) transcription gene regulation (21 SNPs).

Lipid metabolism CAD GRS showed significant associations, per one unit of increased in standard deviation, with carotid plaque Odds Ratio (OR):1.05, (95% Confidence interval (CI) 1.007, 1.09) and prevalent CHD OR:1.10 (1.02, 1.19).

Blood pressure CAD GRS was associated with incidence of CHD, HR: 1.07 (1.02, 1.13).

Inflammation CAD GRS showed significant associations in carotid plaque OR:

1.05 (1.007, 1.09), CAC β: 0.22 (0.11, 0.34), AAC β: 0.13, (0.02, 0.23), ECAC β: 0.13, (0.03, 0.23), incidence of CHD HR:1.08 (1.03, 1.14) and incidence of CV mortality HR:1.11 (1.03, 1.19).

Vascular remodeling CAD GRS was associated with ln cIMT β: 0.004, (0.0007, 0.008), prevalence CHD OR:1.14 (1.06, 1.23), and incidence CHD HR: 1.08 (1.02, 1.13). Neovascular angiogenesis CAD pathway was associated with ln IMT β: 0.004, (0.00004, 0.007), AAC β: 0.10, (0.0004, 0.21), prevalence CHD OR:1.11 (1.03, 1.20) and incidence CHD HR=1.08 (1.03, 1.15).

Nitric oxide-signalling CAD GRS showed significant associations with carotid plaque OR:1.07 (1.03, 1.12) and incident CHD HR=1.07 (1.02, 1.13).

Mitosis proliferation CAD GRS was associated with AAD β: -0.11, (-0.23, -0.0008) and ICAC β: -0.13, (-0.23,-0.04).

Finally, transcription gene regulation CAD GRS showed association in ln PWV β: 0.01, (0.004, 0.015), CAC β: 0.15, (0.03, 0.27), prevalence CHD OR:1.18 (1.10, 1.27), incident ischemic stroke HR=1.11 (1.04, 1.19) and incidence of CV mortality HR=1.10 (1.02, 1.19). (table 2)

Variance explained by global and specific biological pathways of CAD GRS

We computed the coefficient of determination (R^2) to measure the percentage of the variability of the outcome variable (i.e. clinical and subclinical measures of atherosclerosis) explained by the independent variables (i.e. CAD GRS) included in the linear regression models. In case of logistic regression models, we selected pseudo R^2. In the global CAD GRS, the largest R^2 was observed for prevalent CHD (1.02%), followed by CAC (0.58%) and AAC (0.39%), In general, low R^2 values were observed for the majority of phenotypes under study (range 0% to 1.02%) in both model for global CAD GRS and specific biological pathways CAD GRS (Table 3).

Analysis based on CAD GRS global and specific biological pathways quartiles

Quartiles of global CAD GRS and specific biological pathways CAD GRS were calculated as a sensitive analysis for reconfirmation of the previous results.

Significance of the association was lost in global CAD GRS and descending TAD, BP CAD GRS and incidence CHD, inflammation CAD GRS and carotid plaque and AAC, neovascular CAD GRS in ln cIMT and AAC. Finally, CAD GRS mitosis proliferation lost significance in AAD. (Supplemental tables S2-S10).

Discussion

In the present study, we reported an association between a GRS based on 160 genome-wide significant CAD SNPs with prevalent and incident CHD and several subclinical measures of atherosclerosis in various vascular beds mainly including coronary and carotid arteries. Moreover, we did not find an association between the CAD genetic variants with prevalent stroke, PAD, ABI, PWV or with diameter of thoracic and abdominal aorta.

The GRS for CAD was associated with both prevalent and incident CHD in our study.The discovery GWAS for the SNPs included in our GRS were mainly based on cross-sectional and case-control designs. It has been suggested that such study designs might lead to over-representation of SNPs associated with a favorable prognosis after CHD events. Therefore, the SNPs associated with prevalent CHD might not be associated with incident CHD. However, unlike previous reports[18], we found a stronger association for the GRS with both incident CHD and prevalent CHD. This could be due to the fact that previous studies have used a considerable smaller number of genome-wide significant SNPs associated to CAD. Using the most recent GWA findings, the number of genome-wide significant CAD SNPs in our GRS was larger than the number of SNPs included in the previous reports.

Compared to prevalent and incident CHD, the CAD GRS showed no statisticllay significant association with prevalent and incident stroke in our study. While the shared common pathological mechanisms and risk factors for CHD and stroke indicate overlap in the genetic pathways leading to these conditions, the results for genetic studies regarding establishing a similar genetic association structure for these two vascular syndromes are conflicting.[28,29]. The findings of our study might suggest partially distinct mechanisms by which common genetic variants contribute to the risk of CAD and stroke.

In our study, the GRS for CAD showed an association with different subclinical measures of atherosclerosis from various vascular beds. The overall stronger association of the CAD genetic variants with measures of atherosclerosis in coronary and carotid arteries in our study is in line with a stronger correlation

between coronary and carotid atherosclerosis with CAD, highlighting the disparity in contribution of various vascular beds in the disease process[30].

Although atherosclerosis process in different vessels share common genetic pathways, the interaction between the genetic and environmental factors and the intermediate biochemical pathways through which they act might contribute differently to the disease process in various vessels. Moreover, compared to more centrally located arteries, peripheral arterial diseases might compromise more phenotypically heterogenous phenotypes and therefore the genetic susceptibility loci might differ across different subtypes of the disease in different vascular trees[31].

CAD is a highly polygenic disease. LeBlanc et al have recently demonstrated an interesting genetic dissociation among conventional cardiovascular risk factors and CAD, with strong enrichment for lipids, inflammation, and metabolic disorders.[32] Our results showed higher association among transcription gene regulation, inflamamation and vascular remodeling CAD GRS with a number of the clinical and subclinical atherosclerosis phenotypes under study.

In the present study, we did not find any association between a GRS for CAD with diameters of thoracic or abdominal aorta or with arterial stiffness measured by PWV. While abdominal and thoracic aortic aneurysms were historically thought to both arise from atherosclerotic degeneration of the aortic wall, recent evidence suggest that these conditions are distinct disease entities. Salfati et al have reported pathogenetic differences between fatty streaks in the coronary arteries and those in the aorta and have suggested that at least some of the susceptibility loci for CAD might operate exclusively in the coronary tree[34]. Our results regarding no association of a GRS for CAD with diameters of abdominal or thoracic aorta are in line with the recent hypothesis that abdominal and thoracic aortic aneurysms are not simply a manifestation of atherosclerosis, but a separate, although possibly related disease entity[35,36]. PWV is a measure of arterial stiffness that has shown strong correlation with ageing. Lack of association between the GRS for CAD with PWV in our study could also be viewed in line with the hypothesis that aging, not concomitant atherosclerosis, is probably the dominant factor responsible for the increase of PWV[37,38].

Our study has certain strengths and limitations. We examined the association between a GRS comprising the robust genome-wide significant associated SNPs for CAD with both prevalent and incident CHD and stroke as well as a range of subclinical measures of atherosclerosis in various vessels in the same population. A large sample size with available detailed genotype and phenotype information allowed for comparison of these associations. However, our population consisted

entirely of Caucasians aged 55 years and older and therefore our findings may not be generalizable to younger or nonwhite populations.

Conclusions

A genetic risk score based on 160 genome-wide signficant CAD SNPs showed a differential association with clinical CVD events and subclinical measures of atherosclerosis in various vascular beds. These findings could indicate genetic heterogeneity in atherosclerosis process in different vessels and call for better understanding of the genetic structure involved in site-specific atherosclerosis that could contribute to development of more specific diagnostic and therapeutic tools.

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Figure 1 Association of a coronary artery disease genetic risk score, in quartiles, with incident coronary heart disease

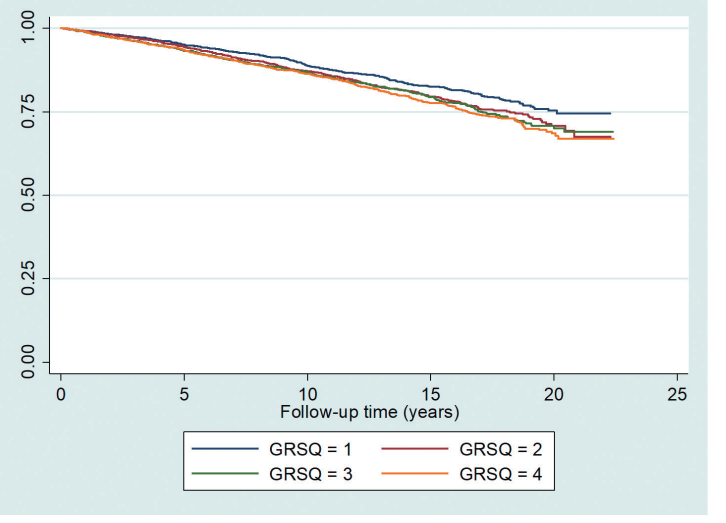


Table 1. Baseline characteristics of 11496 study population

	All (n= 11496)
Cardiovascular risk factors	
Age, years (SD)	65.32 (9.91)
Female (%)	6672 (58.04)
Systolic BP, mmHg (SD)	138.3 (21.6)
Diastolic BP, mmHg (SD)	77.09 (11.95)
Antihypertensive treatment (%)	3452 (30.10%)
Total Cholesterol, mmol/L (SD)	6.17 (1.23)
HDL cholesterol, mmol/L (SD)	1.37 (0.39)
Lipid lowering medication (%)	1108 (9.66%)
BMI, kg/m² (SD)	26.85 (4.07)
Current smoking (%)	2695 (23.83%)
Prevalent DM (%)	1054 (11.93%)
Family history of CVD (%)	4741 (51.67%)
Genetic risk score (SD)	11.04 (0.49)
cIMT, mm (SD)	0.99 (0.20)
Carotid plaque (%)	6633 (66.57%)

	All (n= 11496)
ABI (SD)	1.05 (0.19)
PAD (%)	1248 (16.63 %)
PWV (SD)	12.82 (3.09)
Ascending TAD, mm (SD)	36.4 (3.65)
Descending TAD, mm (SD)	28.7 (2.67)
AAD, mm (SD)	19.09 (2.57)
CAC, (ln+1), median (p25-75)	4.14 (1.25-5.76)
AAC, (ln+1), median (p2-p75)	5.60 (3.92- 6.84)
ECAC, (ln+1), median (p25-75)	3.32 (0-4.86)
ICAC, (ln+1), median (p25-75)	3.88 (2.25-5.05)
Prevalent CHD (%)	326 (2.84%)
Incident CHD (%)	1493 (14.05%)
Prevalent stroke (%)	805 (7.28%)
Incident ischemic stroke (%)	805 (7.56%)
Incident CVD mortality (%)	675 (5.87%)

Values are mean (SD: standard deviation), median (25th - 75th percentile), or numbers (percentages). Abbreviations: AAD, abdominal aortic diameter; ABI, ankle brachial index; BMI, body mass index; BP, blood pressure; CAC, coronary artery calcification; CHD, coronary heart disease; cIMT, carotid intima media thickness; CVD, cardiovascular disease; DM, diabetes mellitus; ECAC, extra-cranial carotid calcification; HDL, high-density lipoprotein cholesterol; ICAC, Intra-cranial carotid calcification; PAD, peripheral arterial disease; PWV, pulse wave velocity; TAD, thoracic aorta diameter.

Table 2. Association of global standardized genetic risk score of CAD and specific biological pathways in clinical and subclinical atherosclerosis

	Global GRS all 160 SNPs	GRS Lipid 17 SNPs	GRS Blood Pressure 7 SNPS	GRS inflammation 15 SNPS	GRS remodeling 23 SNPs	GRS Neovascular 9 SNPs	GRS NOs 7 SNPs	GRS Mitosis 16 SNPS	GRS Transcription 21 SNPs
Subclinical atheroscl.									
Ln cIMT	0.0036 (6.9e-06, 0.007)	0.0006 (-0.003, 0.004)	0.003 (-0.001, 0.0069)	-0.002 (-0.005, 0.002)	**0.004 (0.0007, 0.008)** †	**0.004 (0.00004, 0.007)** †	0.003 (-0.0002, 0.007)	-0.002 (-0.006, 0.0013)	0.002 (-0.002, 0.006)
Carotid plaque *	**1.09 (1.05, 1.14)**‡	**1.05 (1.007,1.09)** †	1.003 (0.96, 1.05)	**1.05 (1.006, 1.09)** †	1.01 (0.97, 1.06)	0.99 (0.96, 1.04)	**1.07 (1.03, 1.12)** ‡	0.98 (0.94, 1.02)	1.02 (0.98, 1.07)
ABI	0.00064 (-0.0038, 0.005)	-0.00001 (-0.005, 0.004)	-0.002 (-0.006, 0.002)	-0.005 (-0.009, -0.0005)	0.0015 (-0.003, 0.006)	-0.002 (-0.007, 0.002)	-0.002 -0.006, 0.003)	0.003 (-0.001, 0.007)	0.0005 (-0.004, 0.005)
Ln PWV	0.0043 (-0.0014, 0.10)	-0.0016 (-0.007, 0.004)	-0.0018 (-0.008, 0.004)	-0.0004 (-0.006, 0.005)	0.003 (-0.003, 0.008)	0.003 (-0.003, 0.009)	0.003 (-0.003, 0.009)	-0.002 (-0.008, 0.004)	**0.01 (0.004, 0.015)**‡
Ascending TAD	-0.049 (-0.21, 0.11)	-0.016 (-0.18, 0.15)	0.005 (-0.15, 0.16)	0.15 (-0.014, 0.31)	-0.05 (-0.21, 0.11)	-0.12 (-0.28, 0.045)	0.08 (-0.08, 0.24)	-0.012 (-0.17, 0.15)	-0.01 (-0.17, 0.15)
Descending TAD	**-0.15 (-0.27, -0.04)**‡	-0.048 (-0.17, 0.07)	-0.067 (-0.18, 0.05)	-0.013 (-0.1, 0.11)	-0.08 (-0.20, 0.04)	-0.025 (-0.14, 0.094)	-0.006 (-0.12, 0.11)	0.99 (0.93, 1.05)	0.05 (-0.07, 0.17)
AAD	-0.11 (-0.22, 0.005)	0.033 (-0.083, 0.15)	-0.06 (-0.17, 0.05)	-0.09 (-0.21, 0.03)	-0.09 (-0.20, 0.03)	-0.07 (-0.19, 0.42)	-0.04 (-0.16, 0.07)	**-0.11 (-0.23, -0.0008)**†	-0.003 (-0.12, 0.11)
CAC	**0.20 (0.088, 0.32)**‡	0.065 (-0.052, 0.18)	-0.05 (-0.17, 0.06)	**0.22 (0.11, 0.34)** ‡	0.05 (-0.07, 0.16)	0.094 (-0.023, 0.21)	0.001 (-0.12, 0.12)	-0.065 (-0.18, 0.05)	**0.15 (0.03, 0.27)**†
AAC	**0.15 (0.045, 0.25)**‡	0.017 (-0.086, 0.12)	0.07 (-0.03, 0.17)	**0.13 (0.02, 0.23)** †	0.08 (-0.03, 0.18)	**0.10 (0.0004, 0.21)** †	0.03 (-0.08, 0.13)	-0.03 (-0.14, 0.07)	0.10 (-0.001, 0.21)
ECAC	**0.14 (0.038, 0.23)**‡	0.026 (-0.073, 0.13)	0.05 (-0.04, 0.15)	**0.13 (0.03, 0.23)** †	0.03 (-0.07, 0.13)	0.04 (-0.06, 0.14)	0.07 (-0.03, 0.17)	-0.07 (-0.17, 0.02)	0.08 (-0.011, 0.19)

	Global GRS all 160 SNPs	GRS Lipid 17 SNPs	GRS Blood Pressure 7 SNPS	GRS inflammation 15 SNPS	GRS remodeling 23 SNPs	GRS Neovascular 9 SNPs	GRS NOs 7 SNPs	GRS Mitosis 16 SNPS	GRS Transcription 21 SNPs
ICAC	**0.11 (0.013, 0.20)‡**	0.05 (-0.042, 0.15)	0.03 (-0.06, 0.13)	0.08 (-0.016, 0.18)	0.07 (-0.03, 0.16)	0.03 (-0.07, 0.12)	0.02 (-0.07, 0.12)	**-0.13 (-0.23, -0.04)‡**	0.08 (-0.016, 0.18)
Clinical disease									
Prevalent CHD	**1.33 (1.23, 1.43) ‡**	**1.10 (1.02, 1.19) †**	1.05 (0.97, 1.13)	1.08 (0.99, 1.16)	**1.14 (1.06, 1.23) ‡**	**1.11 (1.03, 1.20) ‡**	1.04 (0.97, 1.12)	0.99 (0.92, 1.07)	**1.18 (1.1, 1.27) ‡**
Incident CHD	**1.14 (1.08, 1.20) ‡**	1.04 (0.99, 1.09)	**1.07 (1.02, 1.13)‡**	**1.08 (1.03, 1.14)†**	**1.08 (1.02, 1.13)‡**	**1.08 (1.03, 1.15) ‡**	**1.07 (1.02, 1.13) ‡**	1.02 (0.97, 1.07)	1.0009 (0.95, 1.05)
Prevalent ischemic stroke	1.09 (0.98, 1.22)	1.07 (0.96, 1.20)	1.05 (0.94, 1.18)	0.95 (0.85, 1.07)	1.05 (0.94, 1.17)	0.99 (0.88, 1.10)	1.04 (0.93, 1.16)	1.03 (0.92, 1.15)	1.11 (0.99, 1.24)
Incident ischemic stroke	1.05 (0.98, 1.13)	1.02 (0.95, 1.09)	1.003 (0.94, 1.07)	1.02 (0.96, 1.1)	0.99 (0.93, 1.07)	1.06 (0.99, 1.14)	0.95 (0.89, 1.02)	1.007 (0.94, 1.08)	**1.11 (1.04, 1.19) ‡**
Incident CVD mortality	**1.11 (1.03, 1.20) ‡**	1.03 (0.96, 1.11)	1.05 (0.97, 1.13)	**1.11 (1.03, 1.19) ‡**	1.04 (0.97, 1.13)	1.04 (0.96, 1.12)	0.99 (0.91, 1.06)	1.01 (0.94, 1.09)	**1.10 (1.02, 1.19) †**
Prevalent PAD*	0.98 (0.92, 1.04)	0.97 (0.92, 1.036)	1.03 (0.97, 1.09)	1.05 (0.99, 1.12)	0.98 (0.92, 1.04)	1.01 (0.95, 1.08)	1.04 (0.98, 1.10)	0.99 (0.93, 1.05)	0.99 (0.93, 1.05)

Ln cIMT: intima media thickness logarithm, ABI: ankle brachial index, Ln PWV: pulse wave velocity logarithm, TAD: thoracic aorta diameter, AAD: abdominal aortic diameter, CAC: coronary artery calcification, AAC: aortic arch calcification, ECAC: extra-cranial carotid artery calcification, ICAC: intra-cranial carotid artery calcification, CHD: coronary heart disease, CVD: cardiovascular disease.

[1] Odds ratio (95% confidence interval); [2] Beta coefficient (95% confidence interval); [3] Hazard ratio (95% confidence interval).

Bold type: statistical significance † : $p < 0.05$, ‡ : $p < 0.01$

Table 3. Variance explained for each of genetic risk score of global CAD and specific pathways

	GRS all 160 SNPs	GRS Lipid 17 SNPs	GRS Blood Pressure 7 SNPS	GRS inflammation 15 SNPS	GRS remodeling 23 SNPs	GRS Neovascular 9 SNPs	GRS NOs 7 SNPs	GRS Mitosis 16 SNPS	GRS Transcription 21 SNPs
Subclinical measures of atherosclrosis									
Ln cIMT	0.0004	0.0000	0.0002	0.0001	**0.0006 †**	**0.0004 †**	0.0003	0.0002	0.0001
Carotid plaque	**0.0014 ‡**	**0.0004 †**	0.0000	**0.0004 †**	0.0000	0.0000	**0.001 ‡**	0.0001	0.0001
ABI	0.0000	0.0000	0.0001	0.0006	0.0001	0.0001	0.0001	0.0002	0.0000
Ln PWV	0.0004	0.0000	0.0001	0.0000	0.0001	0.0002	0.0002	0.0001	**0.002 ‡**
Ascending TAD	0.0002	0.0000	0.0000	0.0016	0.0002	0.001	0.0004	0.0000	0.0000
Descending TAD	**0.0034 ‡**	0.0003	0.0007	0.0000	0.001	0.0001	0.0000	0.0000	0.0003
AAD	0.0018	0.0002	0.0006	0.0012	0.001	0.0008	0.0003	**0.002 †**	0.0000
CAC	**0.0058 ‡**	0.0006	0.0004	**0.007 ‡**	0.0003	0.0012	0.0000	0.0006	**0.0031 †**
AAC	**0.0039 ‡**	0.0001	0.0009	**0.0027 †**	0.001	**0.0019 †**	0.0001	0.0002	0.0018
ECAC	**0.0036 ‡**	0.0001	0.0006	**0.0031 †**	0.0002	0.0003	0.0009	0.001	0.0015
ICAC	**0.0024 ‡**	0.0006	0.0002	0.0013	0.0009	0.0001	0.0001	**0.004 ‡**	0.0013
Clinical cardiovacular disease									
Prevalent CHD	**0.0102 ‡**	**0.0012 †**	0.0003	0.0007	**0.0004 ‡**	**0.0038 ‡**	0.0002	0.0000	**0.0036 ‡**
Prevalent ischemic stroke	0.0009	0.0005	0.0003	0.0002	0.0002	0.0000	0.0001	0.0001	0.0012
Prevalent PAD*	0.0001	0.0001	0.0001	0.0004	0.0001	0.0000	0.0002	0.0000	0.0000

Ln cIMT: intima media thickness logarithm, ABI: ankle brachial index, Ln PWV: pulse wave velocity logarithm, TAD: thoracic aorta diameter, AAD: abdominal aortic diameter, CAC: coronary artery calcification, AAC: aortic arch calcification, ECAC: extra-cranial carotid artery calcification, ICAC: intra-cranial carotid artery calcification, CHD: coronary heart disease, PAD: peripheral artery disease.

Bold type: statistical significance **† : $p < 0.05$, ‡ : $p < 0.01$**

Supplemental Material

Table S1. SNPs associated with coronary artery disease included in the genetic risk score

Nr	SNP	Gene at locus	Effect allele	Beta
1	rs36096196	ZFPM2	T	0,049
2	rs2493298	PARP12, TBXAS1	A	0,058
3	rs61776719	MAD1L1	A	0,039
4	rs11206510	DHX38, HP, DHODH	T	0,077
5	rs17114036	DNAJC13, NPHP3, ACAD11, UBA5	A	0,157
6	rs599839	SWAP70	A	0,104
7	rs11806316	RP11-664H17.1	G	0,039
8	rs11810571	PRDM8, FGF5	G	0,068
9	rs4845625	KCNK5	T	0,058
10	rs1892094	BCAP29, GPR22	C	0,039
11	rs6700559	MRVl1, CTR9	C	0,039
12	rs2820315	CCDC92	T	0,049
13	rs60154123	SCARB1	T	0,049
14	rs17465637	HNRNPD, RASGEF1B	C	0,131
15	rs699	SVEP1	G	0,039
16	rs515135	HOXC4	C	0,068
17	rs6544713	LRP1, STAT6	T	0,058
18	rs582384	PCSK9	A	0,030
19	rs1561198	LDLR, SMARCA4	T	0,058
20	rs2252641	SERPINA2, SERPINA1	C	0,058
21	rs12999907	TMEM106B, THSD7A	A	0,058
22	rs840616	ZC3HC1, KLHDC10	C	0,039
23	rs6725887	TRIM5, TRIM22, TRIM6, OR52N1, OR52B6	C	0,131
24	rs1250229	COL6A3	T	0,068
25	rs2571445	ANGTPL4	A	0,039
26	rs2972146	MAD2L1, PDE5A	T	0,068
27	rs1801251	NGF, CASQ2	A	0,049
28	rs11677932	TDRKH, RP11-98D18.9	G	0,030
29	rs748431	KSR2	G	0,039
30	rs7633770	C1S	A	0,030
31	rs7617773	TCF21, TARID (EYA4−AS1)	T	0,039
32	rs7623687	CYP17A1, CNNM2, NT5C2	A	0,068
33	rs142695226	ARHGEF26	G	0,077
34	rs10512861	SHROOM3, SEPT11, FAM47E, STBD1	G	0,039
35	rs667920	FN1, ATIC, LOC102724849, ABCA12, LINC00607	T	0,049
36	rs2306374	PHACTR1, EDN1	C	0,113

Nr	SNP	Gene at locus	Effect allele	Beta
37	rs12493885	PCNX3, POLA2, RELA, SIPA1, and others	C	0,068
38	rs4266144	FNDC3B	G	0,030
39	rs12897	Ral1, PEMT, RASD1, SMCR3, TOM1L2	G	0,039
40	rs16844401	ZNF507, LOC400684	A	0,068
41	rs17087335	FIGN	T	0,058
42	rs12500824	MCF2L, PCID2, CUL4A	A	0,039
43	rs10857147	CDKN1A, PI16	T	0,058
44	rs11099493	ANRIL, CDKN2B-AS	A	0,039
45	rs3775058	ARNTL	A	0,039
46	rs11723436	CORO6, BLMH, ANKRD13B, GIT1, SSH2, EFCAB5	G	0,049
47	rs35879803	LIPA	C	0,049
48	rs1878406	UMPS, ITGB5	T	0,095
49	rs7692387	SEMA5A, TAS2R1	G	0,077
50	rs7696431	VAMP5, VAMP8, GGCX	T	0,039
51	rs1508798	CENPW	T	0,049
52	rs3936511	HGFAC, RGS12, MSANTD1	G	0,039
53	rs1800449	PLEKHG1, IYD	T	0,086
54	rs273909	REST, NOA1	G	0,068
55	rs2706399	PPAP2B	G	0,068
56	rs246600	CXCL12	T	0,049
57	rs9501744	MIA3, AIDA, C1orf58	C	0,049
58	rs12526453	FURIN, FES	C	0,095
59	rs17609940	gene desert	G	0,068
60	rs1321309	GOSR2, MYL4, ARL17A, and others	A	0,030
61	rs10947789	ANKS1A, UHRF1BP1	T	0,068
62	rs6905288	TSPAN14, MAT1A, FAM213A	A	0,049
63	rs9367716	LOX	G	0,039
64	rs4613862	CETP	A	0,030
65	rs1591805	KCNJ13, GIGYF2	A	0,039
66	rs12190287	ADORA2A	C	0,077
67	rs17080091	PECAM1, DDX5, TEX2	C	0,049
68	rs3798220	EDNRA	C	0,412
69	rs4252120	ATP1B1, BLZF1, CCDC181, F5, NME7, SELP, SLC19A2	T	0,068
70	rs10267593	SNRPD2, GIPR	G	0,039
71	rs7797644	HDAC9	C	0,039
72	rs11509880	DHX58, KAT2A, RAB5, NKIRAS2, DNAJC7, KCNH4, HCRT, GHDC	A	0,039
73	rs2023938	APOE, APOC1, TOMM40, PVRL2, COTL1	C	0,077
74	rs2107732	CCM2, MYO1G	G	0,058
75	rs10953541	ARID4A, PSMA3	C	0,077
76	rs975722	SMG6, SRR	G	0,030
77	rs11556924	HNF1A, OASL, C12orf43, and others	C	0,086

Nr	SNP	Gene at locus	Effect allele	Beta
78	rs10237377	ZEB2, TEX41	G	0,049
79	rs3918226	MRAS, CEP70	T	0,131
80	rs6997340	ARHGAP26	T	0,039
81	rs264	PRDM16, PEX10, PLCH2, RER1	G	0,100
82	rs6984210	KIAA1462	G	0,077
83	rs10093110	TNS1, CXCR2, RUFY4	G	0,030
84	rs2954029	ZNF831	A	0,058
85	rs1333049	LPL	C	0,255
86	rs944172	IL5, RAD50	C	0,039
87	rs111245230	SLC22A4	C	0,131
88	rs885150	LMOD1, IPO9, NAV1, SHISA4, TIMM17A	C	0,030
89	rs579459	MAP3K7CL, BACH1	C	0,095
90	rs61848342	HHIPL1, YY1	C	0,039
91	rs2505083	TRIB1	C	0,068
92	rs1746048	LOC646736, IRS1, MIR5702	C	0,086
93	rs17680741	SH2B3, FLJ21127, ATXN2, and others	T	0,049
94	rs1412444	ZNF827	T	0,086
95	rs12413409	MORN1, SKI	G	0,113
96	rs4918072	UNC5C	A	0,039
97	rs4752700	LPA, SLC22A3, LPAL2	G	0,030
98	rs11601507	ADAMTS7	A	0,086
99	rs10840293	PCIF1, ZNF335, NEURL2, PLTP	A	0,058
100	rs11042937	CFDP1, BCAR1	T	0,030
101	rs1351525	NOS3	T	0,049
102	rs7116641	MAP3K1, MIER3	G	0,030
103	rs12801636	PLG, LPAL2	G	0,049
104	rs590121	CCNL1, TIPARP	T	0,049
105	rs7947761	FAM46A	G	0,039
106	rs974819	UBE2Z, GIP, ATP5G1	T	0,068
107	rs964184	HTRA1, PLEKHA1	G	0,122
108	rs11838267	COL4A1, COL4A2	T	0,049
109	rs10841443	IL6R, AQP10, ATP8B2, CHTOP, UBAP2L	G	0,058
110	rs11170820	STN1, SH3PXD2A	G	0,095
111	rs11172113	APOB	C	0,058
112	rs7306455	SMAD3	G	0,049
113	rs3184504	ABO, SURF6, GBGT1	T	0,068
114	rs11830157	PRKCE, TMEM247	G	0,113
115	rs2244608	SERPINH1	G	0,058
116	rs11057401	SORT1, PSCR1, CELSR2	T	0,077
117	rs11057830	HHAT, SERTAD4, DIEXF	A	0,068
118	rs9319428	ZHX3, PLCG1, TOP1	A	0,058
119	rs9591012	FHL3, UTP11, SF3A3, MANEAL, INPP5B	G	0,039

Nr	SNP	Gene at locus	Effect allele	Beta
120	rs4773144	CDC123, NUDT5, OPTN	G	0,068
121	rs1317507	OAZ2, RBPMS2, TRIP4, and others	A	0,039
122	rs2145598	ABCG5, ABCG8	G	0,030
123	rs112635299	PMAIP1, MC4R	G	0,122
124	rs2895811	STAG1, MSL2, NCK1, PPP2R3A	C	0,068
125	rs6494488	DDX59, CAMSAP2, KIF14	A	0,049
126	rs56062135	WDR12, CARF, FAM117B, ICA1L, NBEAL1	C	0,068
127	rs3825807	VEGFA, MRPL14, TMEM63B	A	0,077
128	rs8042271	BMP1, SFTPC, DMTN, PHYHIP, DOK2, XPO7	G	0,095
129	rs17514846	AGT, CAPN9, GNPAT	A	0,068
130	rs17581137	NAT2	A	0,039
131	rs1800775	HSD17B12	C	0,030
132	rs1050362	PLCG2, CENPN	A	0,039
133	rs3851738	BCAS3	C	0,068
134	rs7199941	MMP9	A	0,039
135	rs7500448	FCHO1, COLGALT1	A	0,068
136	rs216172	NDUFA12, FGD6	C	0,068
137	rs12936587	FGD5	G	0,068
138	rs13723	CDH13	G	0,039
139	rs76954792	CDC25A, SPINK8, MAP4, ZNF589	T	0,039
140	rs2074158	RHOA, AMT, TCTA, CDHRA, KLHDC8B, and others	C	0,049
141	rs17608766	ALS2CL, RTP3	C	0,068
142	rs46522	GUCY1A1*	T	0,058
143	rs7212798	COPRS, RAB11FIP4	C	0,077
144	rs1867624	PALLD, DDX60L	T	0,039
145	rs9964304	DAGLB, RAC1, FAM220A, KDELR2	C	0,039
146	rs663129	ARHGAP42	A	0,058
147	rs116843064	MFGE8, RP11-326A19.4, ABHD2	G	0,131
148	rs1122608	HNRNPUL1, CCDC97, TGFB1, B9D2	G	0,131
149	rs73015714	CALCRL, TFPI	G	0,058
150	rs12976411	PROCR, ASIP, NCOA6, ITGB4BP/EIF6 and others	A	0,285
151	rs8108632	DAB2IP	T	0,049
152	rs2075650	FLT1	G	0,131
153	rs1964272	PRIM2, RAB23, DST, BEND6	G	0,039
154	rs867186	KLF4	A	0,068
155	rs6102343	FOXC1	A	0,039
156	rs3827066	N4BP2L2, PDS5B	T	0,039
157	rs7270354	APOA1-C3-A4-A5	A	0,058
158	rs260020	PDGFD	T	0,039
159	rs2832227	CTTNBP2, CFTR, ASZ1	G	0,039
160	rs9982601	ACAA2, RPL17	T	0,166
161	rs180803	MRPS6, SLC5A3, KCNE2	G	0,182

Quartile analysis in subclinical and clinical phenotypes in phenotypes with statistical significance differences.

Table S2. Association of GRS-CAD quartiles based on 161 SNPs and cardiovascular outcomes in 11496 participants

GRS-CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
Carotid plaque[1]	Reference	1.13 (1.005, 1.27) †	1.11 (0.99, 1.26)	1.24 (1.10, 1.40) ‡
Descending TAD[2]	Reference	-0.20 (-0.53, 0.13)	-0.009 (-0.34, 0.32)	-0.26 (-0.59, 0.07)
CAC[2]	Reference	0.04 (-0.28, 0.37)	0.34 (0.01, 0.66) †	0.57 (0.25, 0.89) ‡
AAC[2]	Reference	0.20 (-0.09, 0.48)	0.12 (-0.16, 0.41)	0.51 (0.22, 0.80) ‡
ECAC[2]	Reference	0.10 (-0.18, 0.38)	0.10 (-0.17, 0.38)	0.53 (0.26, 0.81) ‡
ICAC[2]	Reference	0.17 (-0.09, 0.43)	0.24 (-0.02, 0.51)	0.41 (0.15, 0.68) ‡
Clinical				
Prevalent CHD[1]	Reference	0.87 (0.69, 1.10)	1.26 (1.01, 1.57) †	1.80 (1.46, 2.21) ‡
Incident CHD[3]	Reference	1.11 (0.96, 1.29)	1.22 (1.05, 1.41) ‡	1.37 (1.19, 1.59) ‡
Incident CVD mortality[3]	Reference	1.15 (0.92, 1.44)	1.21 (0.97, 1.51)	1.37 (1.10, 1.70) ‡

TAD: thoracic aorta descending, CAC: carotid artery calcification, AAA: aortic arch calcification, ECAC: extra cranial carotid artery, ICAC: intra cranial carotid artery, CHD: coronary heart disease, CVD: cardiovascular disease.

[1] Odds ratio (95% confidence interval); [2] Beta coefficient (95% confidence interval); [3] Hazard ratio (95% confidence interval).

Bold type: statistical significance † **: p < 0.05, ‡ : p < 0.01**

Table S3. Association of GRS-CAD quartiles based on 17 SNPs related **with lipid metabolism** and cardiovascular outcomes in 11496 participants

4.3

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
Carotid plaque[1]	Reference	1.09 (0.97, 1.22)	1.09 (0.97, 1.23)	**1.14 (1.01, 1.28)** †
Clinical				
Prevalent CHD[1]	Reference	1.07 (0.86, 1.33)	1.20 (0.97, 1.49)	**1.28 (1.03, 1.58)** †

CHD: Coronary heart disease, [1] Odds ratio (95% confidence interval).

Bold type: statistical significance † **: p < 0.05, ‡ : p < 0.01**

Table S4. Association of GRS-CAD quartiles based on 7 SNPs related with **blood pressure** and cardiovascular outcomes in 11496 participants

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Clinical				
Incidence CHD[3]	Reference	1.01 (0.87, 1.17)	1.07 (0.93, 1.24)	1.15 (0.99, 1.32)

CHD: Coronary heart disease, [3] Hazard ratio (95% confidence interval).
Bold type: statistical significance † **: p < 0.05, ‡ : p < 0.01**

Table S5. Association of GRS-CAD quartiles based on 15 SNPs related **with inflammation** and cardiovascular outcomes in 11496 participants

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
Carotid plaque[1]	Reference	1.001 (0.89, 1.13)	1.08 (0.96, 1.21)	1.12 (0.99, 1.26)
CAC[2]	Reference	0.34 (0.017, 0.67) †	0.12 (-0.20, 0.45)	0.61 (0.29, 0.94) ‡
AAC[2]	Reference	0.03 (-0.26, 0.31)	0.12 (-0.16, 0.41)	0.27 (-0.02, 0.56)
ECAC[2]	Reference	0.10 (-0.18, 0.38)	0.15 (-0.12, 0.43)	0.33 (0.053, 0.61) †
Clinical				
Incident CHD[3]	Reference	1.03 (0.89, 1.19)	1.14 (0.99, 1.32)	1.24 (1.07, 1.43) ‡
Incident CVD mortality[3]	Reference	0.80 (0.64, 1.01)	1.10 (0.89, 1.36)	1.28 (1.05, 1.58) †

CAC: carotid artery calcification, AAA: aortic arch calcification, ECAC: extra cranial carotid artery, CHD: coronary heart disease, CVD: cardiovascular disease. [1] Odds ratio (95% confidence interval); [2] Beta coefficient (95% confidence interval); [3] Hazard ratio (95% confidence interval). **Bold type:** statistical significance † **: p < 0.05, ‡ : p< 0.01**

Table S6. Association of GRS-CAD quartiles based on 23 SNPs related with vascular remodeling and cardiovascular outcomes in 11496 participants

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
Ln cIMT[2]	Reference	0.006 (-0.004, 0.017)	0.008 (-0.003, 0.018)	0.012 (0.002, 0.02) ‡
Clinical				
Prevalent CHD[1]	Reference	1.09 (0.88, 1.36)	0.97 (0.78, 1.22)	1.49 (1.21, 1.83) ‡
Incident CHD[3]	Reference	1.07 (0.93, 1.24)	1.08 (0.93, 1.25)	1.22 (1.06, 1.41) ‡

Ln cIMT: logarithm of intima media thickness, CHD: coronary heart disease, [1] Odds ratio (95% confidence interval); [2] Beta coefficient (95% confidence interval); [3] Hazard ratio (95% confidence interval). **Bold type:** statistical significance † **: p < 0.05, ‡ : P< 0.01**

Table S7. Association of GRS-CAD quartiles based on 9 SNPs related with neovascularization angiogenesis and cardiovascular outcomes in 11496 participants

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
Ln cIMT[2]	Reference	-0.0003 (-0.011, 0.010)	-0.001 (-0.01, 0.009)	0.01 (-0.0007, 0.020)
AAC[2]	Reference	0.09 (-0.20, 0.38)	0.02 (-0.27, 0.31)	0.19 (-0.1, 0.48)
Clinical				
Prevalence CHD[1]	Reference	1.12 (0.90, 1.39)	1.09 (0.87, 1.35)	1.39 (1.12, 1.71) ‡
Incidence CHD[3]	Reference	1.02 (0.88, 1.18)	1.07 (0.93, 1.24)	1.19 (1.03, 1.37) †

Ln cIMT: logarithm of intima media thickness, AAC: aortic arch calcification, CHD: coronary heart disease, [1] Odds ratio (95% confidence interval); [2] Beta coefficient (95% confidence interval); [3] Hazard ratio (95% confidence interval). **Bold type:** statistical significance **† : p < 0.05, ‡ : P< 0.01**

Table S8. Association of GRS-CAD quartiles based on 7 SNPs related with NO-Signalling and cardiovascular outcomes in 11496 participants

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
Carotid plaque[1]	Reference	1.006 (0.90, 1.13)	1.05 (0.93, 1.18)	1.15 (1.03, 1.30) †
Clinical				
Incidence CHD[3]	Reference	1.09 (0.94, 1.27)	1.11 (0.96, 1.29)	1.25 (1.08, 1.44) ‡

CHD: coronary heart disease, [1] Odds ratio (95% confidence interval); [3] Hazard ratio (95% confidence interval). **Bold type:** statistical significance **† : p < 0.05, ‡ : P< 0.01**

Table S9. Association of GRS-CAD quartiles based on 16 SNPs related with mitosis proliferation and cardiovascular outcomes in 11496 participants

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
AAD[2]	Reference	0.017 (-0.31, 0.34)	-0.30 (-0.62, 0.022)	-0.20 (-0.52, 0.12)
ICAC[2]	Reference	-0.30 (-0.56, -0.032)	-0.07 (-0.33, 0.20)	-0.40 (-0.66, -0.14)‡

AAD: abdominal aortic diameter, ICAC: internal carotid artery calcification, [2] Beta coefficient (95% confidence interval).
Bold type: statistical significance **† : p < 0.05, ‡ : P< 0.01**

Table S10. Association of GRS-CAD quartiles based on 21 SNPs related with transcription gene regulation and cardiovascular outcomes in 11496 participants

GRS- CAD quartiles				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
Subclinical				
Ln PWV[2]	Reference	0.003 (-0.013, 0.019)	0.0018 (-0.014, 0.018)	0.024 (0.008, 0.040) ‡
CAC[2]	Reference	0.94 (-0.23, 0.42)	0.14 (-0.19, 0.46)	0.48 (0.16, 0.80) ‡
Clinical				
Prevalence CHD[1]	Reference	1.27 (1.02, 1.59) †	1.21 (0.97, 1.52)	1.60 (1.30, 1.98) ‡
Incident ischemic stroke[3]	Reference	0.99 (0.81, 1.21)	1.13 (0.93, 1.38)	1.24 (1.02, 1.50) ‡
Incident CVD mortality[3]	Reference	1.08 (0.86, 1.35)	1.25 (1.004, 1.55) †	1.26 (1.02, 1.56) †

Ln PWV: logarithm of pulse wave velocity, CAC: coronary artery calcification, CHD: coronary heart disease, CVD: cardiovascular mortality.
[1] Odds ratio (95% confidence interval); [2] Beta coefficient (95% confidence interval); [3] Hazard ratio (95% confidence interval).

CHAPTER 5

General Discussion

This thesis used advanced statistical methods to study the dynamic changes in the structure and function of the cardiovascular system, the associated risk factors, and the impact of these changes on the development of clinical outcomes. Three methodological aspects were the thematic axis of this thesis:

1. The analysis of repeated measures of variables such as electrocardiogram in neonates (Chapter 2.1), changes in echocardiographic variables that evaluate left ventricular diastolic function in adults of the Rotterdam Study (Chapter 2.2) and dynamic changes in systolic blood pressure over time and its association with clinical outcomes in the participants of the Systolic blood PRessure INTervention Trial (SPRINT trial) (Chapter 2.3).

2. The application of advanced statistical methods for the analysis of causal inference such as: i) Propensity score matching, applied in the selection of the best control group in quasi-experiments involving vascular interventions in octogenarians diagnosed with abdominal aortic aneurysm (Chapter 3.A1) and in patients with coronary heart disease undergoing Fractional Flow Reserve (FFR) to guide the best therapy (Chapter 3A.2) and, ii) Mendelian randomization analysis to assess the causal relationship between serum levels of dehydroepiandrostenedione sulfate (DHEAs) and N-terminal type B natriuretic pro-peptide (NT-pro-BNP), hormones directly related to changes in the structure and function of the cardiovascular system associated with heart failure and aging (Chapter 3.B1).

3. Finally, the dynamics changes in thoracic aortic diameters and their associated risk factors (Chapter 4.1), the implication of these changes in the development of clinical events (Chapter 4.2) and, the evaluation of the hypothesis that atherosclerosis is a systemic condition affecting different vascular beds in a similar manner was explored (Chapter 4.3).

1. Methodological studies of longitudinal repeated measurements

1.1 Repeated measures in neonatal electrocardiography

Our study is the first cohort study that followed-up the same cohort of newborns throughout the neonatal period and applied the current sampling rate and bandwidth recommendations of the European Society Cardiology consensus[1]. Our findings showed statistically significant differences in the duration and amplitude of 88% of the electrocardiographic variables and 12% in the wave trajectory, when compared with other reportes[2].

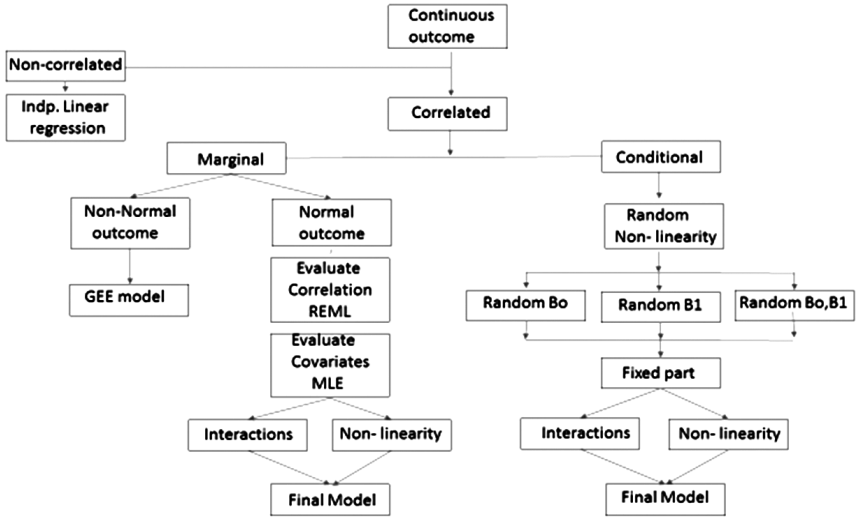
Another novel finding was our description of the association of maternal-fetal factors, with the dynamic changes of the electrocardiographic variables in the neonatal period. For instance, weight at birth was associated with an increase in PR interval in DII and amplitude of Q wave in DIII. Maternal factors such as the number of pregnancies were positively associated with an increased electrical axis and maternal age was positively associated with an increased PR interval. Cesarean section was associated with a significant increase in average QTc interval.

The causes that explain these associations will require other studies. Their implications, in particular the association of cesarean section with the prolongation of the QTc interval which may increase the risk of sudden neonatal death, could be clinically relevant.

To date, the diagnostic criteria of normality of the neonatal electrocardiogram are based on the study of Davignon et al[2], published in 1979. That study is the basis of the current consensus for the diagnosis of the normal neonatal electrocardiogram of the European Society of Cardiology[1]. Unfortunately, even though this study sought to evaluate dynamic changes over time in the neonatal electrocardiogram, it was based on a cross-sectional study including different infants with different postnatal ages. This limits the applicability of its findings. In addition, electrocardiographic records were taken with a low sampling rate and low bandwidth, which could affect the accuracy of the duration and voltage measurements of electrocardiographic waves[3-5].

Our analysis was possible thanks to the application of advanced statistical methods for the analysis of repeated measures such as generalized estimate equation (GEE) model, marginal and mixed linear effect models, which allowed to obtain more precise results, considering the selection of the best matrix of correlation of repeated measurements, the comparison between heterocedastic vs. homocedastic models, and the comparison between models with only random intercept vs models with both intercept and slope random[6-9] (Figure 1).

Figure 1. Flow chart of longitudinal data analysis in neonatal electrocardiogram.



Indp: Independent, GEE: Generalized estimator equation model, REML: Restricted maximum likelihood, MLE: Maximum likelihood estimation, Bo: Beta Intercept, B1: Beta Slope.

Our study has the following strengths:

1. It was a cohort study that followed the same group of newborns during the whole neonatal period with only a small proportion of lost to follow-up (6.7%).

2. We used a digital computerized register according to the current recommendations of sampling rate (1200 HZ) and bandwidth (300 Hz) with standardized location of the electrodes.

3. We had access to an internally validated software to read all waves, segments, and intervals in each ECG.

4. Our results were based on linear regression models for correlated data.

The limitations of this study are:

1. Criteria of cardiovascular normality in newborns under study were based on their (term) gestational age, birth weight, lack of fetal or maternal complications, and clinical evaluation by a neonatologist. Echocardiographic images were not available.

2. Our cohort comprised only Hispanic-American newborns enrolled at a single hospital center, which limits the generalizability of our findings.

3. Neo 1.0 software was internally validated, not externally.

4. While our sample size was sufficient to show significant changes in ECG parameters during the neonatal period, it might have been too small to detect more subtle electrocardiographic abnormalities and limited to adequately assess and address gender differences.

Directions for future research:

1. Multi-center and multi-ethnic studies with electrocardiographic computerized records according to current recommendations[1,5] and available follow-up during the neonatal period in the same cohorts are essential. Such studies would allow for proper statistical analysis of repeated ECG measures as well as disentangling potential sex and racial differences.

1.2 Comparison of repeated measures of left ventricular diastolic function between men and women

Using linear regression mixed effects models[6-9], we found that the progression of six continuous echocardiographic parameters that evaluate left ventricular diastolic function, evolve in a similar way, during 11 years of follow-up, among men and women. However, women were found to have poorer diastolic function than men. Additionallly, we described significant differences in risk factors associated with the progression of diastolic function between men and women. Specifically, diabetes mellitus and BMI were associated with more deterioration in left ventricular diastolic function in men, while smoking was associated with larger deterioration of diastolic function in women. In turn, serum HDL levels were protective only in women.

Cross-sectional studies have previously described that the prevalence of diastolic ventricular dysfunction was two times more frequent in women than in men[10].

In turn, diastolic ventricular dysfunction is a risk factor for the development of heart failure with preserved ejection fraction (HFpEF)[11,12]. Multiple risk factors associated with left ventricular diastolic dysfunction (LVDD) have been described[13,14]. However, there is no published study that analyzed changes in the trajectory of parameters that evaluate diastolic function, and its associated risk factors, between men and women[15].

Our findings generate new hypotheses about differential pathophysiological mechanisms in the progression of left ventricular diastolic function in men and women. The application of advanced statistical methods, such as the linear regression mixed effects models, which allow the analysis of the variability both intra-individual and between men and women, while taking into account the best correlation matrix and the fixed and random effects in the model, has been very helpful to this end.

Our study has the following strengths:

1. It was a cohort study with repeated echocardiograms over 11 year of follow-up.

2. Inclusion of a large group of women and men made it possible to evaluate gender differences.

3. Access to information on a well-defined set of covariates and detailed characterization of the cohort allowed to examine LVDF parameters and their correlates from a gender-specific perspective.

4. We had a standardized protocol used by 4 trained echocardiographers with good inter and intra-reader agreement

The limitations of this study are:

1. The gold standard for measuring diastolic function is the pressure-volume relationship, which is invasive. However, Doppler measurements of mitral inflow and TDI should also allows for a valid non-invasive measurement of diastolic function[16,17]

2. Our population included individuals of European ancestry. Therefore, the generalizability of our findings to other ethnicities should be done with caution.

3. Survival bias cannot be entirely ruled out.

Directions for future research:

1. Clinical implications of diastolic function changes over time in the development of clinical outcomes, including incident heart failure and cardiovascular mortality, must be studied in more detail.

2. JM analysis is a novel statistical method for the simultaneous analysis of repeated measuremen and time to event data. This method could be considered as a useful method in the analysis of left ventricular diastolic function parameters under study.

1.3 Repeated measures of systolic blood pressure in the Systolic Blood Pressure Intervention Trial (SPRINT Trial)

The main aim of the SPRINT trial[18] was to evaluate if an intensive treatment approach (lowering SBP below 120 mmHg) compared with standard treatment (maintaining SBP between 135-139 mmHg) decrease the hazard for the composite SPRINT primary outcome (myocardial infarction, other acute coronary syndrome, heart failure, stroke and cardiovascular mortality). The traditional Cox model analysis was the statistical method used in the original SPRINT trial.

The SPRINT trial measured systolic blood pressure (SBP) monthly in the first trimester and then every quarter for 3.26 years median follow-up (range 0 to 4.5 years) in 9361 participants, achieving an average of 15 SBP measurements per person (range: 1-21) during follow-up. This information was not taken into account in the primary SPRINT analysis. Given that: (i) this clinical trial evaluates a strategy of intensive pharmacological intervention (decreasing the SBP <120 mmHg) versus conventional treatment (SBP between 135-139 mmHg), (ii) blood pressure is among the most important risk factors for major cardiovascular events (primary SPRINT outcome), (iii) there is a high SBP variability within the subjects during follow-up, and (iv) the occurrence of serious adverse events (SAEs) during follow-up can impact both the subsequent SBP levels as well as the primary outcome; a statistical analysis is required that evaluates the impact of longitudinal changes in SBP, both within individuals and between intervention groups, and takes also the cumulative effect of the SBP on the primary outcome into account (Indirect effect) + the effect of this intervention on the primary SPRINT outcome (Direct effect). This analysis can only be achieved with a statistical model that includes all these elements at the same time, such as the cumulative joint model (cJM) analysis (Figure 2)

Figure 2. Cumulative joint model analysis in the SPRINT trial.

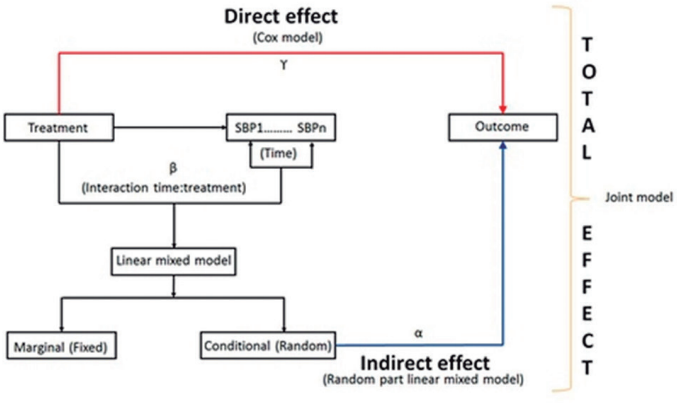


Figure 2. SBP1: First measurement of systolic blood pressure; SBPn: last measurement of systolic blood pressure. (Complete history of all SBP measurements previous to primary SPRINT outcome and its area under the curve (cumulative SBP) are being taken into account in the cumulative joint model analysis)

Using this cumulative joint model approach, we showed that intensive SBP treatment only decreased the risk of the SPRINT primary outcome in the initial phase of follow-up. Unfortunately, this initial protection was lost after 3.4 years of follow-up in the overall SPRINT population and earlier in some subgroups including women, black participants, participants with a history of coronary heart disease or chronic kidney disease, participants younger than 75 years of age and those with SBP higher than 132 mmHg at baseline. The factors that caused the loss of protection were related to the deleterious cumulative effect of maintaining a SPB <120 mmHg for long periods of time, to the high frequency of severe adverse events produced by intensive treatment, and to the significant increase in visit-to-visit SBP variability produced by such intervention.

Our paper led to an editorial comment published simultaneously in the Journal of Hypertension[19] and a Letter to the Editor of the main authors of the SPRINT study[20].

The main concern from Dr. Reboussin and Dr. Whelton to our secondary analysis could be summarized as:

- Analyses of clinical trials that adjust for variables measured after randomization estimate something very different than standard clinical trial analyses which do not employ adjustment or only adjust for baseline variables

- Rueda-Ochoa et al. made a serious error in defining the 'total treatment effect' as one that 'accounts for differences in SBP over time'. In fact, what they report is the component of the intervention effect that excludes the effects produced by changes in SBP. This cannot be viewed as a complete summary of the intervention effect. At best, estimation of this adjusted effect represents a technical accomplishment without clear implications for clinical decision-making.

- The Rueda-Ochoa et al secondary SPRINT analysis is similar to the CASE-J trial reported by Oba paper previously published[21] (Figure 3).

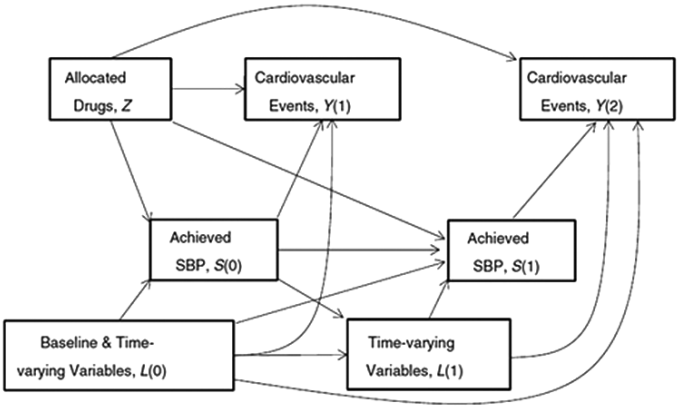


Figure 3 Directed acyclic graph of the CASE-J trial

Compared with the standard intervention, the SPRINT intensive intervention resulted in substantial health benefits, including prevention of CVD events, total mortality, and cognitive impairment that were, if anything, larger later in the follow-up period rather than smaller, as suggested by Rueda-Ochoa et al.

Our Reply to Dr. Reboussin and Dr. Whelton Editorial Letter[22]

- The joint modeling approach does *not* condition on systolic blood pressure (SBP) after randomization but rather treats it as an outcome. In particular, the joint model uses a linear mixed model for SPB that explicitly allows for different SPB profiles in the two treatment groups. In addition, among others, it accounts for the correlations in the repeated SBP measurements per patient,

for missing at random missing data, and the endogenous nature of SPB that relate to the challenges in the interpretation mentioned by Dr. Reboussin and Dr. Whelton.

- The total effect that we reported is the sum of both direct and indirect effect of the intervention. (Figure 2)

- Our cumulative Joint model analysis is not similar to CASE-J trial analysis since a marginal approach was used therein. We used a conditional (mixed) model approach + Cox proportional hazard model = Joint model analysis. For more detail comparison between these approaches, we recommend Lindsey JK and Lambert Paper[23].

- It should be remembered that the cumulative joint model analysis takes into account the effect of SBP (cumulative and intra-individual SBP variability) on primary SPRINT outcome and not the marginal difference in events. Clearly, the differences found in the changes over time in the HR in our analysis compared with the number of events marginally reported by Whelton et al can be along the lines of Yule-Simpson´s paradox.

Joint modeling to time to event data analysis should be considered as a powerful statistical tool to assess the total effect of an intervention, by including in the same analysis both direct and indirect effects, the latter given through a biomarker that is repeated with time, which approaches the conditions encountered in daily clinical practice.

Our study has the following strengths:

1. Randomization was maintained in our statistical analysis.

2. Both direct and indirect effects of intensive treatment were included in the analysis.

3. Initial sudden decline in SBP was taken into account during modelling process.

4. SBP was the outcome in linear mixed model and it was not included as a classical mediator.

5. SBP repeated measurements increase statistical power.

The limitations of this study are:

1. If SAEs were colliders negatively or positively correlated to both treatment and to primary SPRINT outcome, adjusting for them would introduce selection bias towards the null[24]

2. Cumulative joint model analysis is a novel and not frequently considered approach for assessment of clinical trial data.

Directions for future research:

1. Due to the controversial results obtained in this secondary analysis of the SPRINT study, new analyzes are required, using other statistical approaches to validate our results (Triangulation approach). In this sense, Rizopoulos et al have recently published a new analysis of the effect that SAEs have on the outcome of the SPRINT study, using a more flexible joint model but reaching similar conclusions[25].

2. Advanced methods for causal inference

2.1 Selection of control group in quasi-experimental observational studies

Within this thesis, we undertook several steps to select the proper control groups for the two intervention groups under study (chapter 3A.1 and chapter 3A.2). We evaluated different biases and inaccuracies that could rise from different approaches used for control selection, including the impact of time period as well as the traditional age and sex only matching, versus the Propensity Score Matching (Greedy) approach. In the following paragraphs, we detail these steps and discuss the results obtained with each approach.

2.1.1 Evaluation of time period bias on the selection of the control group

The selection of an adequate control group should consider the same time period of observation to the group intervened. In this way, it is ensured that both the control and intervention groups are sharing the same conditions such as the availability of similar medicines and health technologies as well as exposure to common environmental factors such as diet, socioeconomic and cultural conditions, all of which can potentially impact the outcomes under study. Not taking into account

the effect of the observation time period while selecting the control group may produce inaccurate results, which is known in epidemiology as a period or cohort bias[26-28]. In chapter 3A.2, we explored the possibility of this bias during the process of control group selection. To this end, we selected two different control groups from the Rotterdam Study (RS) randomly matched by age and sex with the intervention groups (medical therapy group and revascularization therapy group). The first control group was selected from participants of the RS who were enrolled in the study between 1990-1993. The second control group was selected from participants of the RS who were enrolled between 2000-2008 and at the same time period to the intervention groups. Results of the Cox proportional hazards analysis for comparison of both sets of controls with the medical therapy group, with all-cause mortality as the outcome, are presented in Table 1. As shown in the table, when comparing the medical therapy group compared with a control group enrolled a decade earlier, the survival of the groups did not differ (hazard ratio (HR); 95% confidence interval (CI) for all-cause mortality: 1.15; 0.93, 1.42). Conversely, the risk of all-cause mortality in the MT group was significantly larger than the matched control group from the same enrollment period. (HR; 95%CI: 1.60; 1.28, 2.00). For the revascularization group, the HRs (95% CIs) were respectively 1.17 (0.79, 1.74) and 1.49 (0.98, 2.27) when compared to a control group enrolled a decade earlier and the matched control group from the same enrollment period. (Table 1) This step clearly portraits the phenomenon known as the period or cohort bias. A good approach to minimize this bias is to select the control group from a comparable and similar recruitment period as the intervention group.

Table 1: Hazard ratio for all-cause mortality for comparison of the medical therapy and revascularization groups with control groups from different enrollment periods.

Intervention groups	Control group enrollment period	HR (95% CI)
Medical therapy 2000- 2008	1990 – 1993	1.15 (0.93, 1.42)
Medical therapy 2000- 2008	2000 – 2008	1.60 (1.28, 2.00)*
Revascularization 2000 - 2008	1990 – 1993	1.17 (0.79, 1.74)
Revascularization 2000 - 2008	2000 – 2008	1.49 (0.98, 2.27)

HR: hazard ratio, 95% CI: 95% confidence interval.
* P value for the difference between the medical intervention or the revascularization group and the Rotterdam Study control group < 0.05
Models were additionally adjusted for body mass index, smoking, family history of cardiovascular disease, dyslipidemia, hypertension.

2.1.2 Selection of control group based on age and sex only

The most frequently used method to select control groups is matching only by age and sex. This method, however, is not the ideal method to arrive at a complete balance between the intervention and control groups and reduce confounding, as given other important variables might not be balanced between the two groups. Other unbalanced variables, therefore, should be considered to be included in the models as adjustment variables. However, adjustment for covariates also does not ensure a complete balance between the groups and confounding may still be present in the association under study. In chapter 3A.2, we explored the possibility of biases arising from control group selection based on age and sex matching only.

When comparing the medical therapy and revascularization therapy groups with control groups matched on age and sex only, the HRs (95% CIs) were 1.60 (1.28, 2.00) and 1.49 (0.98, 2.27) respectively. Since age and sex only matching did not lead to full balance between the intervention and control groups, the Cox proportional hazards models were adjusted for other covariates such as body mass index, family history of coronary artery disease, current smoking, and hypertension.

2.1.3 Propensity score matching to use population-based studies as controls for interventional studies

Propensity score matching (PSM) is a statistical matching technique that attempts to estimate the effect of an intervention, including treatment, by accounting for the factors that predict receiving the intervention. PSM approximates to a random trial by matching controls with experimental subjects. Propensity models depend on the potential outcomes, assuming every subject has two potential outcomes: one if they were treated (intervention group), the other if they are not treated (control group). The aim is to compare treated subjects to untreated subjects with the same potential outcomes. The difference between treated and untreated subjects is due to treatment, since the outcomes in both groups would have been the same had the treated subjects not received treatment. Subjects with the same propensity score have, on average, the same potential outcomes. So, comparing treated and untreated subjects with the same propensity score gives a less confounded estimate of the effect of treatment[29-32].

In order to calculate a propensity score and do a matching based on it, all possible reasons based on logic, theory and empirical findings for which the treatment group and the control group would differ (i.e. all potential confounding variables) need

to be taken into account. Of note, all constructs that are theoretically considered as part of the treatment variable (or a strong proxy for the treatment variable) should not be used as a predictor in the calculation of a propensity score. Once the list of predictors for the propensity score model has been identified and before calculating the propensity scores, the collinearity among the predictors need to be examined. Missing values in covariates should be identified. The cause or mechanism of missingness needs to be explored before imputation. Having taken into account these considerations, covariates identified by the researcher as potential confounders are used as predictors of the dichotomous treatment variable in a logistic regression model. The predicted values (probabilities) obtained from this model are the propensity scores. The goodness of fit of the logistic model should be evaluated using Hosmer-Lemeshow test. If the goodness of fit is not achieved, interaction terms between relevant covariates and non-linear terms in continuous variables can be included in predicting propensity scores. In the current thesis, we applied PSM Greedy approach to select proper control groups from the population-based Rotterdam Study (RS) that are perfectly matched to the intervention groups under study. This technique was applied to two research projects in this thesis (Chapter 3A.1 and 3A.2) to obtain a perfect match in all variables included into the comparison.

2.2 Survival in octogenarians after endovascular repair of abdominal aortic aneursysm

In chapter 3A.1, we wanted to evaluate if the survival in a cohort of octogenarians with diagnosis of abdominal aortic aneurysm (AAA) repaired by EVAR, was similar to octogenarians control group selected from population-based Rotterdam Study Cohort without AAA, using a propensity Greedy approach (Table 2). We report, for the first time, that after EVAR, the life expectancy of octogenarians equals that of the matched octogenarians from the Rotterdam Study, provided that they do not develop early postoperative complications. Furthermore, if a complication occurred, octogenarians, had a nearly two-fold increase in long-term mortality compared to the general population octogenarians. Our results suggest that performing EVAR in octogenarians has a long-term beneficial impact on their life-expectancy, given that patients with low susceptibility to peri- and post-operative complications are selected.

Additionally, we showed that cardiac complications occurred more frequently among octogenarians compared with nonoctogenarians after EVAR. Considering that comorbidities of our two groups were similar at baseline, we hypothesize

that the EVAR procedure triggered the occurrence of such complications. Cardiac complications in our study occurred more often than what has been reported in the literature[33-35] and several studies did not find a higher occurrence of MI after EVAR in octogenarians[36-38]. The larger cardiac complication rate in our study is explained by the fact that we used a combined endpoint of atrial fibrillation, heart failure, myocardial infarction and elevated troponin levels. We routinely

measure postoperative troponin levels in all of our patients. Therefore, we were able to identify elevated troponin levels in patients without symptoms and counted these as cardiac complications.

Previous studies have compared survival after EVAR between octogenarinas and younger patients who have a higher life expectancy and less comorbidities[39-42]. Our study is the first to compare long-term survival between EVAR octogenarians with octogenarians from a population-based cohort study paired using a propensity score matching approach.

Finally, current guidelines lack recommendations on treatment regarding to age of patient or risk of complications and they only state that elective AAA repair is not recommended in patients with a limited life expectancy (2 to 3 years)[43]

Our study has the following strengths:

1. Characterization of a well-defined AAA patient group.

2. Availability of detailed information on a variety of risk factors as well as long-term follow-up data for both AAA patients and the control group.

3. Use of PSM to define a matched control group from the general population.

The limitations of this study are:

1. This was a cohort study, not a randomized clinical trial (RCT), so it is not the best design to answer the question whether or not octogenarians have an increased survival after EVAR. For that we should have compared them with octogenarians with an AAA who were not treated with EVAR.

2. Although all patient data were carefully collected prospectively, there is still a chance that relevant characteristics might not have been recorded (residual confounding)

3. This study was performed in a single tertiary referral institution and may

therefore not be applicable to patients treated in other hospitals.

4. Our patient group was small, especially for performing subgroup analyses. Therefore, we might have been underpowered to detect significant smaller differences between the groups.

Directions for future research:

1. Future research should focus on developing algorithms to identify which octogenarians are prone to develop complications and to optimize peri-operative care to lower the chance of occurrence of complications around EVAR treatment.

Table 2. Baseline characteristics before and after propensity score matching for EVAR octogenarians and the Rotterdam Study controls

	Before propensity score matching			**After propensity score matching**		
	EVAR (n=83)	**RS (n= 2212)**	**p-value**	**EVAR (n=83)**	**RS (n=83)**	**p-value**
Age, years	83.02 (3.0)	83.45 (2.9)	0.18	83.02 (3.0)	82.56 (2.5)	0.28
Female gender, n (%)	14 (16.9%)	1350 (61.0%)	<0.001*	14 (16.9%)	16 (19.3%)	0.69
Smoking (ever), n (%)	58 (69.9%)	773 (35.4%)	<0.001*	58 (69.9%)	60 (72.3%)	0.73
Hypertension, n (%)	65 (78.3%)	1811 (84.9%)	0.10	65 (78.3%)	66 (79.5%)	0.85
PAD, n(%)	11 (13.3%)	176 (9.2%)	0.21	11 (13.3%)	10 (12.1%)	0.82
IHD, n(%)	32 (38.6%)	314 (14.4%)	<0.001*	32 (38.6%)	32 (38.6%)	1.00
Stroke, n(%)	15 (18.1%)	177 (8.1%)	<0.01*	15 (18.1%)	14 (16.9%)	0.84
DM, n(%)	15 (18.1%)	393 (19.3%)	0.79	15 (18.1%)	19 (22.9%)	0.44
Cancer, n(%)	19 (22.9%)	280 (13.9%)	0.02*	19 (22.9%)	24 (28.9%)	0.38

2.3 Ten-year survival after FFR-guided strategy in patients with an isolated proximal left anterior descending coronary stenosis.

In chapter 3A.2, we wanted to investigate 10-year survival of patients with an isolated stenosis in the proximal left anterior descending coronary artery (LAD) in whom the treatment strategy was based on Fractional Flow Reserve (FFR). When FFR was >0.80, medical therapy was chosen (MT group, n=564). When FFR was ≤0.80, revascularization therapy was performed (REV group, n= 165). All-cause mortality of the patient groups was compared with two corresponding control groups from the population-based Rotterdam Study using Propensity Score Matching Greedy approach. Tables 3 and 4 show the baseline characteristics of patients in the medical therapy group and revascularizton therapy group and their matched RS controls before and after the PSM Greedy approach respectively. As shown, there was no statistically significant difference in the characteristics between the two interventions groups and their matched controls after PSM approach was applied. The intervention and control groups were perfectly balanced after PSM approach (table 3-4). We found, after 10-year follow-up, that the medical treatment and revascularization groups did not differ significantly for all-cause mortality. In contrast, when compared to their respective control groups, all-cause mortality was significantly higher in the medical therapy group and in the revascularization therapy group than Rotterdam study control groups. Our results extend the findings of the preliminar study showing that FFR strategy is benefit to improve the long-term survival in patients with proximal LAD stenosis.

FFR is based on presure-flow analysis of a stenosis during maximum coronary flow and indicates the impact of a coronary stenosis on the maximum inducible coronary flow and whether it would be susceptible to induce ischaemia during stress. FFR is the gold-standard to guide therapeutic decisions about PCI or medical treatment in intermediate coronary stenosis (lesions between 30-70%).

Multiple studies have underlined the importance of FFR to take medical decisions in patients with coronary artery disease:

- DEFER trial was designed to evaluate the safety and outcomes of deferring stenting in angiographic stenoses (FFR> 0.75) vs percutaneous coronary intervention (PCI)[44] . After 24 months of follow-up, there was not significant statistical differences in the primary outcome of absence of adverse cardiac outcomes between the groups under study, concluded that PCI is not need in patients with FFR > 0.75. A long-term follow-up to 15 years showed not statistical significant differences for death in both previous mentioned groups, but myocardial infarction was lower in the deferring group compared with PCI group[45].

- The FAME study[46] showed that FFR-guided therapy (revascularization of lesions with a FFR < 0.80) compared with angiographic conventional method (revascularization of lesions with > 50% stenosis) in patients with stable coronary artery disease and multivessel disease was effective in decreasing the primary endpoint (a composite of death, myocardial infarction and repeated vascularization at 1 year follow-up (13.2% versus 18.3% p<0.02). Additionally, FFR strategy reduced the number of stents, decrease the amount of contrast and radiation time, resulting to decrease the procedure costs. These results were maintained up to 2 years after procedure. From 2 to 5 years, the risks of both groups were similar. Thus the FAME trial demonstrated that functional rather than anatomical revascularization should become the standard in this group of patients.

- The FAME II trial[47] was designed to clarify whether PCI of only Haemodynamic significant stenoses (i.e lesions with FFR <0.80) combined with optimal medical therapy would be superior to optimal medical therapy alone as a first approach in patients with stable symptoms. Results showed highly significant difference in the incidence rates of the primary endpoint (cumulative incidence of death, MI or urgent revascularization) between the PCI group and the medical therapy group. The overall conclusion was that lesions with a positive FFR of < 0.80 need to be revascularised upfront rather than started on optimal medical therapy.

Our results indicate that in patients with a proximal LAD stenosis, survival is similar when ischemia producing lesions are alleviated, but that patients with documented coronary atherosclerosis have a significantly higher mortality at 10 years than a population without known coronary artery disease but with the same risk profile. Taken together these findings suggest that the presence of coronary atherosclerosis is a major determinat of survival, more than the presence of ischemia.

Our study has the following strengths:

1. The vast majority of patients were followed-up at the outpatient clinic of the center, their data centralized in the local database and controlled by dedicated research nurses.

The limitations of this study are:

1. The study patients were not randomized

2. Selection of the patients was based on the visual estimation of the proximal LAD stenosis as well as on the clinical context: “typicality” of the complaints,

presence of a previous MI, presence of non-invasevely assessed ischemia, risk of the revascularization procedure, global LV function, among others. While this reflects daily clinical practice, this leaves room for some subjectivity. One should therefore bear in mind that the results cannot be extrapoled to patients with angiographically tight stenosis.

3. The vast majority of patients in the medical therapy group were stable (94.2%). The conclusions of the study can therefore not be extrapolated to patients with acute coronary syndromes. While waiting for the resuts of prospective trials in acute coronary syndromes, it should be reminded that FFR measurements are not routinely recommended in potentially culprit stenoses of acute coronary syndromes.

Directions for future research:

1. Our results suggest that the presence of atherosclerosis, more than of ischemia, is the major determinant of outcomes. This observation merit new investigations.

2. The performance of FFR measurements require be contrasted with quantitative flow ratio (QFR), a novel method for assessing the functional significance of intermediate coronary stenosis without the use of a coronary guidewire.

Table 3: Baseline characteristics of patients in the medical therapy group and their matched Rotterdam Study controls before and after the Propensity Score Matching Greedy approach **(Controls free of prevalence and/or incidence of CHD)**

Characteristics	Medical therapy group	Control group	Mean difference	Medical therapy group	Control group	Mean difference	Bias reduction (%)
	Before propensity score matching			After propensity score matching			
Age, years, mean (SD)	69.11 (7.52)	67.79 (9.38)*	0.273	69.15 (7.53)	69.00 (7.40)	0.019	- 93
Sex (men), N (%)	247 (52.89)	2671 (37.38)*	-0.288	242 (52.49)	226 (49.02)	-0.069	- 76
Dyslipidemia, N (%)	274 (58.67)	2844 (39.83)*	0.322	271 (58.79)	269 (58.35)	0.009	- 97
BMI, kg/m^2 (SD)	26.87 (4.51)	27.46 (4.28)*	-0.141	26.87 (4.51)	26.55 (4.28)	-0.024	- 83
Family history of CHD, N (%)	26 (5.57)	1827 (27.37)*	-0.643	24 (5.21)	32 (6.94)	-0.073	- 89

Characteristics	Medical therapy group	Control group	Mean difference	Medical therapy group	Control group	Mean difference	Bias reduction (%)
	Before propensity score matching			After propensity score matching			
Smoking, N (%)	162 (34.69)	4797 (68.09)*	-0.704	162 (35.14)	147 (31.89)	0.069	- 90
Hypertension, N (%)	238 (50.96)	4283 (63.32)*	-0.215	236 (51.19)	242 (52.49)	-0.026	- 88
N of participants	467	7146	-	461	461	-	-

N= Numbers; SD= Standard deviation; (%) percentage; BMI= Body Mass Index; CHD= Coronary heart disease

* P value for the difference between the medical intervention group and the control group < 0.01

Table 4: Baseline characteristics of patients in the revascularization group and their matched Rotterdam Study controls before and after the Propensity Score Matching Greedy approach **(Controls free of prevalence and/or incidence of CHD)**

Characteristics	Revascu-larization group	Control group	Mean difference	Revascu-larization group	Control group	Mean difference	Bias reduction (%)
	Before propensity score matching			After propensity score matching			
Age, years, mean (SD)	67.60 (7.6)	68.79 (9.38)	0.095	67.62 (7.57)	67.82 (9.05)	- 0.024	- 75
Sex (men), N (%)	100 (75.19)	2671 (37.38)*	-0.802	32 (25.00)	31 (24.22)	0.018	- 98
Dyslipidemia, N (%)	75 (56.39)	2844 (39.83)*	0.244	70 (54.69)	67 (52.34)	0.047	- 81
BMI, kg/m^2 (SD)	27.09 (4.02)	27.46 (4.28)	-0.095	27.11 (4.04)	27.52 (4.27)	- 0.099	4
Family history of CHD, N (%)	10 (7.52)	1827 (27.37)*	-0.604	8 (6.25)	9 (7.03)	- 0.031	- 95
Smoking, N (%)	56 (42.11)	4797 (68.09)*	-0.535	55 (42.97)	54 (42.19)	0.016	- 97
Hypertension, N (%)	71 (53.38)	4283 (63.32)*	-0.168	69 (53.91)	65 (53.91)	0.000	- 100
N of participants	133	7146	-	128	128	-	-

N= Numbers; SD= Standard deviation; (%) percentage; BMI= Body Mass Index; CHD= Coronary heart disease

* P value for the difference between the revascularization intervention group and the control group < 0.01

1.2 Mendelian randomization analysis of DHEAs and NT-Pro-BNP

Our results show for the first time an inverse causal relationship between DHEAs and NT-Pro-BNP, this association being more robust in women compared to men. We also found two common metabolic pathways for DHEAS and NT-Pro-BNP related to inflammation and immunity, which merit additional studies to assess their potential role as pharmacological targets.

Both DHEAs, the most abundant human androgen, and NT-Pro-BNP, are hormones related to changes in the structure and function of the cardiovascular system. Studies have reported that serum levels of DHEAs in men decrease with aging, which in turn has been associated with a greater decrease in left ventricular ejection fraction and a greater risk of cardiovascular events and death. In women, a greater increase in the serum levels of DHEAs and other androgen hormones has been observed after menopause in relation to a decrease in estrogen levels[48].

NT-Pro-BNP is the inactive metabolite of BNP, and its concentrations are highly correlated. In the normal range, the BNP concentration exerts a beneficial cardioprotective effect given its natriuretic role, stimulator of nitric oxide release, its vasodilator, anti-fibrotic and anti-angiogenic effect. On the contrary, very low values of this hormone are related to a loss of its beneficial physiological effects to the heart and blood vessels, as well as an excess in its concentrations are associated with acute myocardial infarction and decompensated heart failure. This excess in its serum concentrations is related to a compensatory response to myocardial dysfunction in the context of heart failure. Under physiological conditions, the serum levels of NT-Pro-BNP are higher in women than in men[48].

Multiple observational studies have reported that there is an inverse relationship between DHEAs and NT-Pro-BNP, finding that this relationship is stronger in women than in men, explaining that lower levels of DHEAs in women compared to men would explain the higher concentration of NT-pro-BNP in women. However, these findings are contradictory. Mendelian Randomization is a method of statistical analysis that allows evaluating causal relationships in observational studies.

The challenges for an adequate Mendelian randomization analysis are multiple and include:

- Have a high quality variable instrument (F statistics> 10)

- Comply with assumptions in relation to the instrumental variable only being

associated with the outcomes only through the exposure factor and not directly

- The instrumental variable is not associated with any covariate that is potentially confusing of the relation between exposure and outcome

- That there are no pleotrophic effects of the instrumental variable with other phenotypes that in turn can be related to the outcome

Our results are robust, especially in the analysis in women given by a strong instrumental variable (F statistics> 10), consistency in the results of the crude analysis and adjusted by covariates, compliance with all assumptions of the model and evidence of no existence of pleiotropia.

Our study has the following strengths:

1. This is the first study to examine the causal association between DHEAs and NT-proBNP levels in a large population-based sample of men and women free of CVD.

2. DHEA and DHEAs were measured using chromatography-tandem mass spectrometry, which is currently considered to be the gold standard method.

The limitations of this study are:

1. In the RS, serum BNP levels were not measured, but solely its inactive precursor NT-proBNP. However, recent systematic reviews and meta-analyses demonstrated that both BNP and NT-proBNP have similar diagnostic and prognostic accuracy in CVDs[49]

2. There were no publically available SNPs on DHEA, therefore, we were notable to calculate GRS of DHEA and we could not study the causal association between DHEA and NT-proBNP. Also, within the RS we did not identify any SNPs associated with serum DHEA. However, DHEAs is the more stable sulfate ester of DHEA, and it can be converted back to DHEA by steroid sulfatase, which can be considered a good proxy of the association between DHEA and NT-proBNP as confirmed in our regression analysis (cross-sectional associations between DHEA and DHEAs and NT-proBNP were in line).

3. In our study, the strength of the GRS as IV measured by the F statistic was satisfactory overall and in females; in men F statistics was close to 10 indicating that in this group, DHEAS GRS might be a weak instrument. However, we consider this could be due to the more robust association of androgens with

NT-pro-BNP levels in women than men, as previously reported[50], and low power, as we confirmed in the power calculation analysis.

Directions for future research:

1. Future clinical studies should investigate potential health benefits from modifying serum DHEAs levels in subjects with heart failure.

2. A bi-directional Mendelian randomization component could also be added to ascertain the possibility of reverse causation. This should be possible because there are previous MRs assessing the health effect of NT-ProBNP on health.

3. Epidemiological studies of aortic structural and function

3.1 Sex-specific distributions and determinants of thoracic aortic diameters in the elderly.

The thoracic and abdominal aortic diameters increase with age, reaching in some individuals the category of aneurysmal dilatations. Risk factors associated with such dilatation have been described, mainly at the level of abdominal aorta. Data about the distribution of thoracic aortic diameters among older persons are scarce and few studies have explored sex differences in the risk factors associated with the increase in aortic diameters mainly at the thoracic level.

Our results (chapter 4.1), using a multivariable linear regression analysis, showed that older age, larger height and weight, higher diastolic blood pressure, lower systolic blood pressure, larger volume of calcifications in coronary arteries and aortic arch and the use of blood pressure-lowering medication were associated with larger absolute ascending and descending aortic diameters. Conversely, the presence of diabetes and the use of lipid modifying agents were associated with smaller ascending and descending aortic diameters. A smaller hip circumference was specifically associated with a smaller ascending aortic diameters. Male sex, current smoking, alcohol consumption and lower HDL cholesterol were specifically associated with larger descending aortic diameters. None of the interaction terms between the potential determinants and sex was significant.

Aditionally, we provide new sex-specific distributions of absolute and BSA-corrected thoracic aortic diameters in middle aged and elderly. For the ascending and descending aorta, the mean diameters in men were 38 mm and 30 mm, and 35 mm and 27 mm in women. An ascending aortic diameter of larger than 40 mm was found in 304 participants (12.1%, 228 men) among whom 6 (0.24%, 4 men)

had a diameter of 55 mm or larger. For the descending aorta, a diameter of 35 mm or higher was found in 49 participants (2.0%, 37 men), with a maximum of 43 mm. Although the difference in mean aortic diameter between the age group of 55-65 years and ≥75 years was only 1 mm for both the ascending and descending aorta, the aortic diameter above 55 years still increased. Our results show the 95th percentile for persons above the age of 75 years old to be 43 mm for ascending and 35 mm for descending aorta for men and 41 mm and 33 for women, an age group for whom distribution data are currently scarce.[51]

The definition of the threshold for thoracic aortic dilatation remains a topic of debate. The current guidelines from the European Society of Cardiology (ESC)[52] define thoracic aortic dilatation as an absolute aortic diameter of larger than 40 mm. This definition of aortic dilatation is that this threshold is derived from cohort-data of relatively young individuals with an age range from 9 to 59 years.[53] This threshold of 40 mm may not be directly applicable to older individuals, especially given increasing evidence suggesting that aortic diameters change with age.[54-56]

From our data, it appeared that absolute values are substantially larger for men than for women, yet BSA-corrected values are larger for women than for men. This suggests that differences in body measures partly explain sex differences in aortic diameters, but that there is still a remaining sex-difference, which results in a larger BSA-indexed values for women. Therefore, we conclude that distribution values should be provided for men and women separately, even when correcting for weight, height or BSA.

Thoracic aortic aneurysms are less prevalent in women as compared with men, but the consequences are worse for women. Female patients with an aortic dissection have a worst surgical outcome and die more frequently than men[57].

Our results suggest the need to reassess current used reference values for thoracic aortic diameters, considering older age and sex.

Our study has the following strengths:

1. The population-based setting and the relatively large sample size. By including participants with hypertension or a history of CVD, we measured the aortic diameter in the general population and not only in healthy people. Therefore the results can be generalised to a larger proportion of the older population.

The limitations of this study are:

1. All thoracic aortic diameters were obtained by the use of non-contrast ECG-gated CT scans. The use of contrast-enhanced CT would have made the measurements even more accurate. However, the use of contrast in predominantly healthy people from the general population is unethical and can cause unnecessary complications, such as an allergic reaction to contrast fluid.

2. Our population was Caucasian which limits the extrapolation of our results to other ethnic groups.

Directions for future research:

1. Multi-ethnic and multi-center studies are requried to validate our results.

2. Future research into the prognostic implications of thoracic aortic diameters and its association with major adverse cardiovascular outcomes is required. This was our main aim for the next chapter of this thesis (chapter 4.2).

3.2 Thoracic aortic diameters and major adverse cardiovascular outcomes. The Rotterdam Study

Following the same cohort of participants evaluated in chapter 4.1, we wanted to assess the independent association between crude, indexed and dichotomous cutoff points (corresponding to 75th to 95th percentiles) of ascending (AA) and descending (DA) thoracic aortic diameters with cardiovascular outcomes as incident coronary heart disease, incident heart failure, incident stroke, cardiovascular mortality and total mortality, among women and men of the Rotterdam study cohort, after 13 year follow-up (chapter 4.2).

For AA diameters, we found that when analyzed continuously, larger crude AA diameter showed a significant association with stroke in only men. Larger crude and height-indexed AA diameters showed a significant association with incident of HF among women for crude and for height-indexed diameter. Weight-indexed and BSA-indexed AA diameters showed a significant association with cardiovascular mortality in only women. Weight-indexed and BMI-indexed AA diameters showed a significant association with all-cause mortality among both women for weight-indexed and for BMI-indexed and men for weight-indexed and for BMI-indexed.

For DA diameters, we found that there was a stronger association between larger crude and indexed DA diameters with the risk of stroke, HF, cardiovascular mortality and all-cause mortality in women than men. Among men, only weight-indexed, BSA-indexed and BMI-indexed DA diameters were associated with all-cause mortality.

For the ascending aorta, a crude diameter ≥ 41 mm (95th percentile) was associated with increased risk for CHD and HF among women and a crude AA diameter ≥ 40 mm (75th percentile) was associated with increased risk for stroke among men as well as the cutoffs based on the 80th, 85th and 90th percentile. For descending aorta in women, crude diameter ≥ 31 mm (95th percentile) was associated with stroke and a crude diameter ≥ 29 mm (75th percentile) was associated with HF together with the cutoffs based on the 80th, 85th and 90th percentiles. A crude diameter ≥ 30 mm (85th percentile) was associated with cardiovascular mortality as well as the cutoffs based on the 90th and 95th percentile. In men, crude DA diameter ≥ 31 mm (75th percentile) was associated with stroke.

Finally, findings from the competing risk analyses showed significant associations for crude and all indexed DA diameters with cardiovascular mortality in women and for weight-indexed and BMI-indexed DA diameters with non-cardiovascular mortality in men. Neither crude nor indexed AA diameter showed any significant associations with cardiovascular or non-cardiovascular mortality among women or men in these analyses

Few studies have shown an association between aortic root diameters and major adverse cardiovascular outcomes. Lam et al[58] assessed association between aortic root and incident of heart failure in the Framingham Heart Study. They found significant association for both baseline and change over time of aortic root diameters and incident HF after adjust for multiple CVD risk factors.

Gondrie et al[59] showed that AA and DA diameters were borderline associated (HR: 1.002 and HR: 1.04 respectively) with a composite outcome of fatal and non-fatal CVD events, after adjused by only age, sex and CT indication.

Conversely, Qazi et al[60] in Participants from the Framingham Offspring and Third Generation Cohorts did not find significant association between dichotomous cut-offs of AA and DA diameters and a composite outcome of CVD death, myocardial infarction, coronary insufficiency, index admission for heart failure, and stroke.

More recent, Kamimura et al[61] report that greater proximal ascending aortic diameter was associated with an increased risk of cardiovascular events in a community-based cohort of blacks.

To our knowledge, our study is the first to determine the associations between ascending and descending aortic diameters with multiple cardiovascular outcomes among women and men separately. While both crude and indexed descending aortic diameters were associated with mortality, the associations for ascending aorta were stronger for indexed values. This suggests that it is important to adjust the ascending aortic diameters for body measurements to adequately predict cardiovascular outcomes. The importance of indexed values was also illustrated by Cuspidi et al[62], who showed that the aortic root indexed by height, but not by BSA or crude diameters, were associated with the composite outcome of fatal or non-fatal cardiovascular events.

We concluded, within the large population-based Rotterdam Study, that we found gender differences in the association of thoracic aortic diameters with the risk of cardiovascular outcomes during 13 years of follow-up. The gender differences were more pronounced for descending aorta, as the descending aortic diameters were strongly associated with stroke, HF, cardiovascular mortality in women. The risk for several cardiovascular outcomes increased significantly at cutoff points that were below the 95th percentile of the distribution of aortic diameters. In addition, we demonstrated that the risk for several cardiovascular outcomes significantly increases at a cutoff point below the sex-specific 95th percentiles of the population distribution. This implies that not only patients with aortic dilatation (aortic diameter >95th percentile) are at risk for major adverse outcomes, but a large group of individuals with mildly enlarged aortic diameters in the general population are at increased risk for cardiovascular outcomes and mortality.

Our study has the following strengths:

1. Our study included the population-based setting and the relatively large sample size which made the sex-specific analyses with multiple outcomes possible.
2. Our results were consistent using continous, standardized and cut-off percentiles of thoracic aortic diameters.

The limitations of this study are:

1. As previous mentioned, The use of contrast-enhanced CT would have made the measurements even more accurate. However, it could be increase the risk of allergies and other adverse effects in a healthy population.
2. The Rotterdam study consists of participants of 55 years or older and predominantly from an European ethnicity. This might limit generalization of our results to younger and non-European populations

Directions for future research:

1. Multicenter and multi-ethnic studies that evaluate the association of thoracic aortic diameters with major adveerse cardiovascular outcomes are required.

2. Repeated measurements analysis of thoracic aortic diameters and its association with clinical outcomes using a JM approach is desiered.

3.3 Polygenic risk score for coronary artery disease and its association with atherosclosis in other vascular beds.

Atherosclerosis is a disease that until now has been considered with potential involvement in different vascular beds, giving it the cognition of being systemic[63]. Epidemiological studies have shown that patients with carotid atherosclerotic disease are at greater risk of suffering from simultaneous coronary heart disease[64,65] Also in the context of diabetic patients, simultaneous multivascular compromise has been observed more frequently, affecting various vascular beds such as coronary arteries, peripheral arteries and brain vascular beds[66].

Although epidemiological evidence strengthens the hypothesis of the vascular systemic compromise of atherosclerosis, there is still no solid and definitive evidence of a common genetic risk profile that supports the systemic hypothesis of atherosclerosis, on the contrary multiple GWAS show specific genetic variants for each of the vascular atherosclerotic phenotypes[67-69].

Given the clear evidence of an association between atherosclerosis of neighboring vascular beds, such as the example of carotid atherosclerosis and coronary heart disease[64], the hypothesis of a possible genetic homunculus is proposed, in which there is a genetic organization responsible for explaining commitment regional atherosclerotic.

Under this hypothesis, a study was conducted that seeks to evaluate the association of a specific polygenic risk score for coronary heart disease, from 160 SNPs with statistical significance reported in GWAS, with subclinical measures of atherosclerosis including common carotid intima media thickness (cIMT) and carotid plaque, ankle brachial index (ABI), pulse wave velocity (PWV), ascending thoracic aorta diameter, descending thoracic aorta diameter, abdominal aortic diameter (AAD), coronary artery calcification (CAC), aortic arch calcification (AAC), extra-cranial carotid artery calcification (ECAC) and intra-cranial carotid artery calcification (ICAC). Also, we assessed the association of the GRS with

prevalent and incident coronary heart disease (CHD), stroke and incident CVD mortality. Moreover, we assessed the association of eight specific GRS for CAD, representative of different biological pathways, with all phenotypes under study.

Our results showed that global CAD GRS has significant associations with carotid plaque, coronay artery calcification, aortic arch calcification, extra-cranial carotid artery calcification, intra-cranial carotid artery calcification, prevalent CHD, incident CHD and CVD mortality. There was no association of global CAD GRS with intima media thickness, ankle-brachial index, pulse wave velocity, ascending/descending thoracic and abdominal aortic diameters, prevalent and incident stroke, and prevalence of peripherical artery disease (PAD). Transcription, inflammation and remodeling were the biological pathways more frequently associated with phenotypes under study.

The CAD GRS showed association with prevalent and incident CAD but not association with prevalent and incident stroke in our study which did not reach statistical significance. While the shared common pathological mechanisms and risk factors for CHD and stroke indicate overlap in the genetic pathways leading to these conditions, the results for genetic studies regarding establishing a similar genetic association structure for these two vascular syndromes are conflicting.[70, 71]. The findings of our study might suggest partially distinct mechanisms by which common genetic variants contribute to the risk of CAD and stroke.

Although atherosclerosis process in different vessels share common genetic pathways, the interaction between the genetic and environmental factors and the intermediate biochemical pathways through which they act might contribute differently to the disease process in various vessels. Moreover, compared to more centrally located arteries, peripheral arterial diseases might compromise more phenotypically heterogenous phenotypes and therefore the genetic susceptibility loci might differ across different subtypes of the disease in different vascular trees[72]

Salfati et al have reported pathogenetic differences between fatty streaks in the coronary arteries and those in the aorta and have suggested that at least some of the susceptibility loci for CAD might operate exclusively in the coronary tree[73]

Recently, we have published a paper[74] that showed that genes such as CCDC7IL and PRKAR2B, located on chromosome 7, are associated with carotid plaque, cIMT and stroke but non-CHD. These genes are colocalized and share similar biological behavior, also showing a specificity for atherosclerotic involvement.

In conclusion, our study showed a stronger association of the CAD genetic variants with measures of atherosclerosis in coronary and carotid arteries but not in other vacular regions, highlighting the disparity in contribution of various genes in atherosclerosis in different vascular beds.

Our study has the following strengths:

1. A large sample size with available detailed genotype and phenotype information allowed for comparison of the association between GRS for CAD with both prevalent and incident CHD and stroke as well as a range of subclinical measures of atherosclerosis in various vessels in the same population.

2. GRS for CAD was based on 160 SNPs published in recent GWAs, increasing the statisticaal power in our study.

The limitations of this study are:

1. Our population consisted entirely of Caucasians aged 55 years and older and therefore our findings may not be generalizable to younger or nonwhite populations.

2. Experimental studies to disentangle the biological pathways involved in the heterogenous compromise of atherosclerosis are mandatory.

Directions for future research:

1. With the recent publication of GWAs for stroke and for peripheral vascular disease, the possibility of evaluating new GRS of these diseases in the study population of the Rotterdam study arises, in order to validate our results.

2. A similar study, in a larger population and with a greater number of outcomes, is required to validate our results.

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CHAPTER 6

Appendices

Summary

Papers in this thesis are presented in three chapters. Chapter 2 focusses on methodological studies of longitudinal repeated measurements. Three papers contribute to this chapter. The first one is based on a cohort of 120 healthy neonates born in a public hospital in Bucaramanga Colombia, who were investigated three times during their first month after birth. The aim of this study was to evaluate the dynamic changes over time in the electrocardiogram, as a reflection of the adaptive changes in structure and function of the cardiovascular system after birth, and to identify the risk factors from the mother, the newborn and the type of delivery associated with these changes. Novel methods like marginal, mixed and GEE models were used in this analysis. Main findings of this paper were: Weight at birth was associated with an increase in average PR interval in DII and average amplitude of Q wave in DIII. Maternal factors such as the number of pregnancies were positively associated with an average increased electrical axis and maternal age was positively associated with an average increased PR interval. Cesarean section was associated with a significant increase in average QTC interval in V3R. This paper is presented in chapter 2.1.

Chapter 2.2 included 870 women and 630 men from the Rotterdam Study cohort, with repeated echocardiographic measurements of left ventricular diastolic function during 11 years of follow-up. Aim of this study was to evaluate sex-differences in risk factors associated with changes over time in left ventricular diastolic function parameters. The framework of linear mixed model was used in this analysis. Smoking among women and metabolic factors (DM and BMI) among men showed larger deleterious associations with longitudinal changes in LVDF parameters. The favorable association of HDL was mainly observed among women.

The third paper of this chapter included 9068 hypertensive adult patients with high cardiovascular risk profile, enrolled in the systolic blood pressure intervention trial (SPRINT trial) in 102 medical centers from United States of America, followed for a median follow-up of 3.26 years. The aim of this secondary analysis was to evaluate the overall effect of the intensive treatment vs the standard treatment approach over the primary SPRINT outcome taking into account both the direct effect of

this intervention, through a traditional Cox proportional hazard analysis, and the indirect effect, through the changes over time in the intra-individual trajectories of the systolic blood pressure and its cumulative effect in all participants. Cumulative Joint model was the novel method used in this analysis. We found that intensive SBP treatment lowered the risk for the primary SPRINT outcome at the start of follow-up. However, the initial beneficial effect was lost during follow-up in the overall population and particularly among participants with prevalent CKD or CVD, women, black individuals, younger participants, those with baseline SBP >132 mmHg, and patients who suffered SAEs. Cumulative SBP, higher SBP variability and SAEs could outweights initial benefit of intensive SBP treatment. This paper is presented in chapter 2.3 and it is followed by two editorial letters (chapter 2.3.1 to 2.3.2).

Chapter 3 described advanced methods for causal inference. This chapter included three papers. The first presented in chapter 3A.1, included 475 non-octogenarians and 83 octogenarians patients from vascular department of Erasmus University Medical Center with diagnosis of abdominal aortic aneurysm under endovascular aortic repair (EVAR) who were followed for 13 years. Main aim of this study was to evaluate 13-years mortality after EVAR in octogenarians compared with octogenarians of the general population without abdominal aortic aneurysm selected from 2212 Rotterdam Study cohort participants using a propensity score matching approach. The second aim was to compare clinical characteristics and complications after EVAR between non-octogenarians and octogenarians of this fourth cohort. Main findings were that after uncomplicated EVAR, octogenarians had a similar survival compared to subjects from the general population (Rotterdam Study cohort), selected using a propensity score matching approach. However, after complicated EVAR, their mortality risk increased two folds. We concluded that patient selection and meticulous peri-operative care is key to avoid decreased long-term survival in octogenarians after EVAR.

Chapter 3A.2, the second paper of chapter 3, included 729 patients from the Invasive Cardiology Department of Aalst hospital in Belgium, with intermediate stenosis in the proximal left anterior descendent (LAD) coronary artery, in whom treatment strategy was based on Fractional Flow Reserve (FFR) measurements. When FFR was ≤0.80, revascularization was performed (REV group, n= 165). When FFR was >0.80, medical therapy was chosen (MT group, n=564). Rates of all-cause death, myocardial infarction (MI), and target vessel revascularization (TVR) were followed up during 10 years. All-cause mortality of both groups was compared with two corresponding control groups without known CAD at baseline from the population-based Rotterdam Study using Propensity Score Matching Greedy

approach. We found that in patients with an isolated stenosis in the proximal LAD, medical therapy for FFR-negative stenosis and revascularization of FFR-positive stenosis were associated with similar survival rates. Yet, regardless of treatment strategy, patients with an isolated LAD stenosis had a significantly higher all-cause mortality than their matched controls without known CAD at baseline.

Chapter 3B.1 was based on observational evidence indicating an inverse association between the levels of the most abundant hormone in the human body, dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAs) and N-terminal pro B-type natriuretic peptide (NT-ProBNP). In this paper, we aimed to generate estimates of the associations of DHEAs (exposure) with NT-proBNP in 7,390 men and women free of cardiovascular diseases from the prospective population-based Rotterdam Study using a Mendelian randomization approach. After the evaluation of all Mendelian randomization assumptions, we found an inverse causal association between DHEAs and NT-proBNP. This study suggests a new metabolic pathway linking DHEAs with NT-proBNP. Our results should stimulate future research to evaluate the potential role of DHEAs in prevention and management of chronic heart failure.

Chapter 4 is focused on epidemiological studies of aortic structure and function. This chapter include three papers from Rotterdam Study cohort participants. In the first two papers (chapter 4.1, 4.2), we evaluated the distribution of ascending and descending thoracic aortic diameter in the elderly population of the Rotterdam Study cohort and the risk factors associated with these diameters among women and men. At the same time, the association of ascending and descending thoracic aortic diameters with major adverse cardiovascular outcomes, such as stroke, coronary heart disease, heart failure, cardiovascular and total mortality were evaluated. In chapter 4.1, we provided novel sex-specific distributions of thoracic aortic diameter for the middle-aged and elderly general population with a high (12.1%) prevalence of AA diameters larger than 40mm. Sex was independently associated with descending aortic diameters, which indicates that sex-specific distribution values should be employed, even when correcting for BSA or height. Traditional cardiovascular risk factors were important determinants of both AA and AD diameters. In chapter 4.2, our study suggested a role for descending aortic diameter as a marker for increased cardiovascular risk for HF, stroke and cardiovascular mortality, in particular among women. The cutoff points for increased risk for several of cardiovascular outcomes were below the 95th percentile of the distribution of aortic diameters.

In chapter 4.3, the hypothesis that atherosclerosis is a unique global condition

mediated by a common genetic profile was evaluated using a coronary artery disease (CAD) genetic risk score based on 160 SNPs reported in most recent GWAS. This genetic risk score (GRS) was the main exposure of the association with clinical and subclinical atherosclerosis outcomes in different vascular beds. Global CAD GRS showed significant associations with carotid plaque, CAC, AAC, ECAC, ICAC, prevalent CHD, incident CHD and incident CVD mortality. There was no association of global CAD GRS with IMT, ABI, PWV, ascending thoracic and abdominal aortic diameters, prevalent and incident stroke, and prevalent of peripheral artery disease (PAD). Transcription, inflammation and remodeling were the biological pathways most frequently associated with phenotypes under study. We concluded that the genetic predisposition for CAD showed a differential association with clinical CVD events and subclinical measures of atherosclerosis in various vascular beds. These findings could indicate genetic heterogeneity in the atherosclerosis process in different vascular beds.

Finally, in chapter 5 a general discussion about the main findings and methodological considerations on the research described in this thesis are presented.

SAMENVATTING

The artikelen in dit proefschrift worden gepresenteerd in drie hoofdstukken. Hoofdstuk 2 richt zich op methodologische studies van longitudinale herhaalde metingen. Drie artikelen dragen bij aan dit hoofdstuk. Het eerste artikel is gebaseerd op een cohort van 120 gezonde neonaten geboren in een openbaar ziekenhuis in Bucaramanga in Colombia. De kinderen werden driemaal onderzocht tijdens de eerste maand na de geboorte. Het doel van deze studie was om de dynamische veranderingen in het elektrocardiogram over tijd te evalueren als een reactie van de adaptieve veranderingen in structuur en functie van het cardiovasculaire systeem na de geboorte, en het identificeren van risicofactoren van de moeder, de pasgeborene en het type bevalling die geassocieerd zijn met deze wijzigingen. Nieuwe methoden zoals marginale, gemengde en GEE-modellen werden gebruikt in deze analyse. De belangrijkste bevindingen van dit artikel waren: geboorte gewicht was geassocieerd met een toename van het gemiddelde PR-interval in DII en de gemiddelde amplitude van de Q-golf in DIII. Maternale factoren zoals het aantal zwangerschappen waren positief geassocieerd met een gemiddeld verhoogde elektrische as en de leeftijd van de moeder was positief geassocieerd met een gemiddeld verhoogd PR-interval. Een keizersnede was geassocieerd met een significante toename van het gemiddelde QTC-interval in V3R. Dit artikel wordt gepresenteerd in hoofdstuk 2.1.

In hoofdstuk 2.2 zijn 870 vrouwen en 630 mannen van de Rotterdam Studie cohort opgenomen, met herhaalde echocardiografische metingen van de linker ventrikel diastolische functie gedurende 11 follow-up jaren. Het doel van deze studie was om geslachtsverschillen te evalueren in risicofactoren die geassocieerd zijn met veranderingen over tijd in de linker hartkamer diastolische functieparameters. Het raamwerk van lineair gemengd model werd gebruikt in deze analyse. Roken bij vrouwen en metabole factoren (T2D en BMI) bij mannen toonden grotere schadelijke associaties met longitudinale veranderingen in LVDF-parameters. De gunstige associatie van HDL werd voornamelijk waargenomen bij vrouwen.

Het derde artikel van dit hoofdstuk bevatte 9068 hypertensieve volwassen patiënten met een hoog cardiovasculair risicoprofiel die deelnamen aan de systolische bloeddrukinterventiestudie (SPRINT trial) in 102 medisch centrums uit de Verenigde Staten van Amerika, gevolgd over een mediaan van 3,26 jaar. Het doel van deze secundaire analyse was het evalueren van het algehele effect van de intensieve behandeling versus de standaard behandelingsaanpak over de primaire uitkomst van SPRINT, rekening houdend met zowel het directe effect van deze interventie via een traditionele Cox-analyse voor proportionele gevaren en het indirecte effect door de veranderingen over tijd in de intra-individuele trajecten

van de systolische bloeddruk en het cumulatieve effect ervan bij alle deelnemers. Cumulative Joint model was de nieuwe methode die in deze analyse werd gebruikt. We vonden dat een intensieve SBP behandeling het risico voor de primaire SPRINT uitkomst aan het begin van de follow-up verlaagde. Het aanvankelijke gunstige effect ging echter verloren tijdens de follow-up in de algehele populatie en in het bijzonder bij deelnemers met prevalent CKD of CVD, vrouwen, Afrikaans Amerikaanse afstammelingen, jongere deelnemers, deelnemers met baseline SBP> 132 mmHg en patiënten met SAE's. Cumulatieve SBP, hogere SBP variabiliteit en SAE's zouden het oorspronkelijke voordeel van een intensieve SBP-behandeling kunnen overtreffen. Dit artikel wordt gepresenteerd in hoofdstuk 2.3 en volgt voor twee redactionele brieven (hoofdstuk 2.3.1 tot 2.3.2).

Hoofdstuk 3 ging over vergevorderde methoden voor causale gevolgtrekkingen. Dit hoofdstuk omvatte drie artikelen. Het eerste artikel omvatte 475 niet-octogene en 83 octogene patiënten van de vaatafdeling van het Erasmus Universitair Medisch Centrum met de diagnose van abdominaal aorta-aneurysma bij endovasculaire aortaherstel (EVAR) met een follow-up periode van 13 jaar. Het hoofddoel van deze studie was de evaluatie van de 13-jarige mortaliteit na EVAR in octogenen vergeleken met octogenen van de algemene populatie zonder abdominaal aorta-aneurysma geselecteerd uit 2212 deelnemers van de Rotterdamse cohort studie met behulp van een propensity score matching methode. Het tweede doel was om klinische kenmerken en complicaties na EVAR te vergelijken tussen niet-octogenen en octogenen van deze cohort. Belangrijkste bevindingen waren dat, na ongecompliceerd EVAR, octogenen een vergelijkbare overleving hadden in vergelijking met de controle groep in de algemene populatie (Rotterdamse studie cohort), maar na een gecompliceerde EVAR nam hun sterfterisico met twee keer toe. We concluderen dat patiënten selectie en zorgvuldige peri-operatieve zorg van cruciaal belang is om te voorkomen dat de langetermijnoverleving bij octogenen na EVAR afneemt. Dit artikel wordt gepresenteerd in hoofdstuk 3A.1.

Hoofdstuk 3A.2, met betrekking tot het tweede artikel van hoofdstuk 3, omvatte 729 patiënten van de afdeling Invasieve Cardiologie van het Aalst-ziekenhuis in België, met intermediaire stenose in de proximale left anterior descendent (LAD) kransslagader, bij wie de behandelingsstrategie was gebaseerd op Fractional Flow Reserve (FFR) metingen. Wanneer FFR $\leq$ 0,80 was, werd revascularisatie uitgevoerd (REV-groep, n = 165). Wanneer FFR > 0,80 was, werd medische therapie gekozen (MT-groep, n = 564). De percentages van overlijden door alle oorzaken, myocardiaal infarct (MI) en revascularisatie van geselecteerde bloedvaten (TVR) werden gedurende 10 jaar opgevolgd. Mortaliteit door alle oorzaken van beide groepen werd vergeleken met twee overeenkomstige controlegroepen zonder

bekende CAD bij aanvang van de op de populatie-gebaseerde Rotterdam-studie met behulp van de Propensity Score Matching Greedy methode. We vonden dat bij patiënten met een geïsoleerde stenose in de proximale LAD, medische therapie voor FFR-negatieve stenose en revascularisatie van FFR-positieve stenose geassocieerd zijn met vergelijkbare overlevingspercentages. Maar ongeacht de behandelingsstrategie hebben patiënten met een geïsoleerde LAD stenose een significant hogere sterfte door alle oorzaken dan hun overeenkomstige controles zonder bekende CAD bij baseline.

Hoofdstuk 3B.1 is gebaseerd op observaties die wijzen op een omgekeerde associatie tussen de niveaus van het meest voorkomende hormoon in het menselijk lichaam, dehydroepiandrosteron (DHEA) en zijn sulfaatester (DHEAs) en N-terminaal pro B-type natriuretisch peptide (NT -ProBNP). Ons doel was om de associaties van DHEA's (blootstelling) met NT-proBNP te testen in 7.390 mannen en vrouwen zonder hart- en vaatziekten van de prospectieve populatie-gebaseerde Rotterdamse studie met behulp van een Mendeliaanse randomisatiebenadering. Nadat alle aannames van Mendeliaanse randomisatie correct waren geëvalueerd, vonden we een omgekeerd causaal verband tussen DHEA's en NT-proBNP. Deze studie suggereert een nieuwe metabole route die DHEA's met NT-proBNP verbindt. Onze resultaten zouden toekomstig onderzoek moeten stimuleren om de potentiële rol van DHEA's bij de preventie en behandeling van chronisch hartfalen te evalueren.

Hoofdstuk 4 is gericht op epidemiologische studies naar de structuur en functie van de aorta. Dit hoofdstuk bevat drie artikelen waarin we gebruik maken van de Rotterdam Studie cohort deelnemers. In de eerste twee artikelen (hoofdstuk 4.1, 4.2) evalueren we de verdeling van de stijgende en dalende thoracale aortadiameter bij de oudere populatie van de Rotterdamse studiecohort en de risicofactoren die geassocieerd zijn met deze diameters in vrouwen en mannen. Tegelijkertijd werd de associatie van oplopende en afnemende Thorax-aortadiameters met belangrijke ongunstige cardiovasculaire uitkomsten, zoals beroerte, coronaire hartziekte, hartfalen, cardiovasculaire en totale mortaliteit geëvalueerd. In hoofdstuk 4.1 geven we nieuwe geslacht specifieke verdelingen van thoracale aortadiameter voor de gemiddelde bevolking van middelbare leeftijd en ouderen met een hoge prevalentie (12,1%) van AA-diameters groter dan 40 mm. Geslacht werd onafhankelijk geassocieerd met afnemende aortadiameters, wat aangeeft dat geslacht specifieke distributiewaarden moeten worden verstrekt, zelfs na correctie voor BSA of lengte. Traditionele cardiovasculaire risicofactoren waren belangrijke determinanten van zowel AA- als AD-diameters. In hoofdstuk 4.2 suggereren de resultaten van de studie een rol voor de dalende aortadiameter als een marker voor verhoogd cardiovasculair risico op HF, beroerte en cardiovasculaire mortaliteit, in

het bijzonder bij vrouwen. De afkappunten voor verhoogd risico op verschillende cardiovasculaire uitkomsten lagen onder het 95e percentiel van de verdeling van de aortadiameters.

In hoofdstuk 4.3 werd de hypothese dat atherosclerose een unieke globale ziekte gemedieerd door een gemeenschappelijk genetisch profiel is, geëvalueerd met behulp van een genetische risicoscore op basis 160 SNP's geassocieerd met coronaire hartziekte zoals gerapporteerd in de meest recente GWAS. Deze genetische risicoscore was de belangrijkste blootstelling in de associatie met klinische en subklinische resultaten van atherosclerose in verschillende vaatbedden. Globale CAD GRS vertoonde significante associaties met carotis plaque, CAC, AAC, ECAC, ICAC, prevalente CHD, incidente CHD en incidente cardiovasculaire mortaliteit. Er was geen associatie van globale CAD GRS met IMT, ABI, PWV, stijgende thoracale en abdominale aortadiameters, prevalente en incidente beroerte en prevalentie van perifere arterieziekte (PAD). Transcriptie, ontstekingen en her-modellering waren de biologische routes die frequenter geassocieerd waren met de onderzochte fenotypes. We concludeerden dat de sterk genetische aanleg voor CAD een differentiële associatie aantoonde met klinische CVD-events en subklinische metingen van atherosclerose in verschillende vaatbedden. Deze bevindingen zouden kunnen wijzen op genetische heterogeniteit in het atherosclerose proces in verschillende vasculaire vaten.

Ten slotte wordt in hoofdstuk 5 een algemene discussie gegeven over de belangrijkste bevindingen en methodologische overwegingen over het onderzoek beschreven in dit proefschrift.

Publications

1. The cardiovascular risk profile of middle age women previously diagnosed with Premature Ovarian Insufficiency: a case-control study. Marlise N. Gunning, Cindy Meun, Bas B. van Rijn, Nadine M.P. Daan, Jeanine E. Roeters van Lennep, Yolande Appelman, Eric Boersma, Leonard Hofstra, Clemens G. K. M. Fauser, **Oscar L. Rueda-Ochoa**, Mohammad A. Ikram, Maryam Kavousi, Cornelis B. Lambalk, Marinus J.C. Eijkemans, Joop S.E. Laven, Bart C.J.M. Fauser. Plos One 2020;

2. Epigenetic link beween statin therapy and type 2 diabetes. Carolina Ochoa-Rosales, Eliana Portilla-Fernandez, Jana Nano,Rory Wilson, Benjamin Lehne,Pashupati P. Mishra, Xu Gao, Mohsen Ghanbari, **Oscar L. Rueda-Ochoa**, Diana Juvinao-Quintero, Marie Loh, Weihua Zhang, Jaspal S. Kooner,Hans J. Grabe, Stephan B. Felix,Ben Sch¨ottker, Yan Zhang,Christian Gieger,Martina M¨uller-Nurasyid,Margit Heier, Annette Peters, Terho Lehtim¨aki, Alexander Teumer, Hermann Brenner,Melanie Waldenberger, M. Arfan Ikram, Joyce B.J. van Meurs, Oscar H. Franco, Trudy Voortman, John Chambers, Bruno H. Stricker, and Taulant Muka. Diabetes Care 2020; 43: 1-9. https://doi.org/10.2337/dc19-1828.

3. Echocardiographic parameters, speckle tracking, and brain natriuretic peptide levels as indicators of progression of indeterminate stage to Chagas cardiomyopathy. Luis E. Echeverría MD, Lyda Z. Rojas, María C. Villamizar, Carlos Luengas, Angel M. Chaves, 3 | Jaime A. Rodríguez, Rafael Campo, Claudia Clavijo, Adriana M. Redondo, Luis A. López, Sergio Alejandro Gómez-Ochoa, Carlos A. Morillo, **Oscar L. Rueda-Ochoa**, Oscar H. Franco. Echocardiography 2020; 00: 1- 10. DOI: 10.1111/echo.14603

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5. The cardiovascular risk profile of middle-aged women with polycystic ovary syndrome. Meun C, Gunning MN, Louwers YV, Peters H, Roos-Hesselink J, Roeters van Lennep J, **Rueda Ochoa OL**, Appelman Y, Lambalk N, Boersma E, Kavousi M, Fauser BC, Laven JS; CREW consortium. Clin Endocrinol (Oxf). 2019 Oct 22. doi: 10.1111/cen.14117.

6. Sex-specific distributions and determinants of thoracic aortic diameters in the elderly. Bons LR, **Rueda-Ochoa OL**, El Ghoul K, Rohde S, Budde RP, Leening MJ, Vernooij MW, Franco OH, van der Lugt A, Roos-Hesselink JW, Kavousi M, Bos D. Heart. 2020 Jan;106(2):133-139. doi: 10.1136/heartjnl-2019-315320. Epub 2019 Sep 24.

7. Development and verification of prediction models for preventing cardiovascular diseases. Sung JM, Cho IJ, Sung D, Kim S, Kim HC, Chae MH, Kavousi M, **Rueda-Ochoa OL**, Ikram MA, Franco OH, Chang HJ. PLoS One. 2019 Sep 19;14(9):e0222809. doi: 10.1371/journal.pone.0222809. eCollection 2019.

8. 10-Year Survival After FFR-Guided Strategy in Isolated Proximal Left Anterior Descending Coronary Stenosis. Milkas A, **Rueda-Ochoa OL**, Fournier S, Muller O, Van Rooij F, Franco OH, Collet C, Barbato E, Kavousi M, De Bruyne B. J Am Coll Cardiol. 2019 Sep 10;74(10):1420-1421. doi: 10.1016/j.jacc.2019.07.013.

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Leebeek FW, de Maat MP. J Thromb Haemost. 2016 Nov;14(11):2114-2120. doi: 10.1111/jth.13479. Epub 2016 Oct 3.

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2. Fellowship American College of Physicians, ACP.
3. Director Electrocardiography Research Group. Universidad Industrial de Santander, Bucaramanga, Colombia.
4. Member European Society of Cardiology ESC
5. Member American Heart Association AHA
6. Member American College of Physicians ACP
7. Member Colombian association of Internal Medicine ACMI
8. Member of Society for Epidemiologic Research SER

Erasmus MC PhD portafolio

Summary of PhD training

PhD student:	Oscar Leonel Rueda-Ochoa
Erasmus MC Department:	Epidemiology
Research School:	Netherlands Institute of Health Sciences (NIHES)
PhD Period:	August 2015- August 2019
Promotor:	Prof.dr. Jaap W. Deckers
	Prof.dr. Dimitris Rizopoulos
	Prof.dr. Oscar H. Franco
Copromotor:	Prof.dr. Maryam Kavousi

1. TRAINING

General academic skill courses	**Year**	**ECTS**
Study Design (CC01)	2015	4.3
Biostatistical Methods I: Basic principles (CC02)	2015	5.7
Develop Research proposal	2015	2.5
Biostatistical Methods II: Classical Regression Models (EP03)	2016	4.3
Oral Research Presentation (PRES)	2017	1.4
English Language ((SC01)	2017	1.4
Introduction to Medical writing	2017	2.0
Research Period PIN health sciences (PIN-RP)	2015-2017	29.6
MSc. In Genetic Epidemiology		
Principles of Research in Medicine and Epidemiology (ESP01)	2016	0.7
Principles of Genetic Epidemiology (ESP43)	2016	0.7
Genomics in Molecular Medicine (ESP57)	2016	1.4
Advances in Genomic Research (ESP63)	2017	0.4
Genetic Epidemiology Research Methods (GE02)	2016	5.1
Advances in Genome-Wide-Association Studies (GE03)	2016	1.4
Family Based Genetic Analysis (GE05)	2017	1.4
SNPs and Human Diseases (GE08)	2016	1.4
An introduction to Analysis of next-Generation Sequencing Data (GE13)	2017	1.4
Linux for Scientists (GE14)	2016	0.6
Human Epigenomics	2017	0.7

Genome-Wide-Association Studies (ESP74)	2016	0.7
Scientific integrity	2019	
Elective Courses		
Repeated Measurements in Clinical Studies (CE08)	2016	1.4
Methods of Clinical Research (ESP10)	2016	0.7
Methods of Public health Research (ESP11)	2016	0.7
Logistic Regression (ESP66)	2017	1.4

Other activities

1. PAPERS PRESENTED IN INTERNATIONAL CONGRESS

European Society Cardiology Congress 2016 (Rome, Italy):

Fire Poster Oral Presentation:

OL. Rueda Ochoa, PL Trigos, VM Mora, LZ Rojas Sanchez, SC Coba, A. Coy, FA Rueda, OH Franco M. Kavousi. Longitudinal data analysis of neonatal electrocardiogram. European Heart Journal, Volume 37, Issue suppl_1, 24 August 2016. Abstract 2136. https://doi.org/10.1093/eurheartj/ehw432

European Society Cardiology Congress 2017 (Barcelona, Spain):

Advances in Science, Oral Presentation:

5959 Changes in systolic blood pressure over time as predictor of major cardiovascular events: a joint model analysis of the SPRINT trial

O.L. Rueda-Ochoa, L.Z. Rojas Sánchez, O.H. Franco, J. Deckers, D. Rizopoulos, M. Kavousi

European Heart Journal, Volume 38, Issue suppl_1, 1 August 2017, ehx493.5959, https://doi.org/10.1093/eurheartj/ehx493.5959

Poster:

P3413 Prevalence of cardiac abnormalities in Chagas disease in the general population: a systematic review and meta-analysis

L.Z. Rojas Sanchez, M. Glisic, L. Pletsch, **O.L. Rueda-Ochoa**, L.E. Echeverria ...

European Heart Journal, Volume 38, Issue suppl_1, 1 August 2017, ehx504. P3413, https://doi.org/10.1093/eurheartj/ehx504.P3413

Poster:

P4307 Associations of endogenous estradiol and testosterone levels with plaque composition and risk of stroke in subjects with carotid atherosclerosis

M. Glisic; B. Mujaj; **O.L. Rueda-Ochoa**; E. Asllanaj; J.S.E. Laven; M. Kavousi; M.W. Vernooij; M.A. Ikram; D. Bos; O.H. Franco; T. Muka

European Heart Journal, Volume 38, Issue suppl_1, 1 August 2017, ehx504. P4307, https://doi.org/10.1093/eurheartj/ehx504.P4307

American College of Cardiology 2017 (Washington, USA)

Poster:

Sex differences in risk factors for longitudinal changes in left ventricular diastolic function: the Rotterdam study

Oscar L. Rueda-Ochoa, Marco A. Smiderle Gelain, Jaap Deckers, Dimitris Rizopoulos, Klodian Dhana, Albert Hofman, Oscar H. Franco, Maryam Kavousi

Journal of the American College of Cardiology March 21, 2017, 69 (11 Supplement) 886; DOI: 10.1016/S0735-1097(17)34275-4

European Society Cardiology Congress 2018 (Munich, Germany):

Poster moderate oral presentation:

P6039 Diameters of the thoracic aorta and their association with mortality in the general population

O L Rueda Ochoa; L R Bons; S Rohde; K El Ghoul; R P J Budde; M W Vernooij; O H Franco; A Van Der Lugt; J W Roos-Hesselink; M Kavousi; D Bos;

European Heart Journal, Volume 39, Issue suppl_1, 1 August 2018, ehy566. P6039, https://doi.org/10.1093/eurheartj/ehy566.P6039

Poster:

P4548 Diameters of the thoracic aorta: Gender-specific references ranges and association with body size and atherosclerotic factors

L R Bons; **O L Rueda Ochoa**; S Rohde; K El Ghoul; R P J Budde; M W Vernooij;

O H Franco; A Van Der Lugt; J W Roos-Hesselink; M Kavousi; D Bos.

European Heart Journal, Volume 39, Issue suppl_1, 1 August 2018, ehy563. P4548, https://doi.org/10.1093/eurheartj/ehy563.P4548

Published: 28 August 2018

Poster:

P3649 Evaluating the 10-year survival after an FFR-guided strategy in patients with proximal isolated stenosis in the left anterior descending coronary artery: impact of control selection

O L Rueda Ochoa; A N Milkas; S Fournier; O Muller; G Cicarrelli; P Xaplanteris; F Van Rooij; M A Ikram; E Wyffels; M Vanderheyden; J Bartunek; O H Franco; E Barbato; B De Bruyne; M Kavousi

European Heart Journal, Volume 39, Issue suppl_1, 1 August 2018, ehy563. P3649, https://doi.org/10.1093/eurheartj/ehy563.P3649

American Heart Association Congress 2018 (Chicago, USA)

Poster:

Abstract 15083: Impact of Cumulative Systolic Blood Pressure and Serious Adverse Events on Efficacy of Intensive Blood Pressure Treatment: A Randomized Clinical Trial

Oscar L Rueda-Ochoa, Lyda Z Rojas Sanchez, Shahzad Ahmad, Cornelia M van Duijn, M. Arfan Ikram, Jaap W Deckers, Oscar H Franco, Dimitris Rizopoulos, and

Maryam Kavousi

Circulation. 2018; volumen 138, Suppl_1:A15083

Caritas International Congress 2018 (London, UK)

Poster:

Mortality in octogenarians after endovascular aortic repair of an abdominal aneurysm: fourteen years of follow-up. **Oscar L. Rueda-Ochoa**[1,2*], Pieter van Bakel[3*], Sanne Hoeks[3], Hence Verhagen[3], Jaap Deckers[1], Dimitris Rizopoulos[1], M. Arfan Ikram[1], Ellen Rouwet[3], Klaas Ultee[3], Sander ten Raa[3], Oscar H. Franco[1,4],

Maryam Kavousi[1**], Marie Josee van Rijn[3**]

European Society Cardiology Congress 2019 (Paris, France):

Descending aortic thoracic diameter: a risk marker for major adverse cardiovascular outcomes in women. **OL Rueda Ochoa**, L.R Bons, S Rohde, K. El Ghoud, MK Ikram, JW Deckers, MW Vernooij, OH Franco, A. van Der Lugt, JW Roos-Hesselink, D. Bos, M. Kavousi.

Intensive blood pressure treatment significantly increases visit-to-visit systolic blood pressure variability. A randomized clinical trial. **OL Rueda-Ochoa**, LZ Rojas Sanchez, MA Ikram, JW Deckers, OH Franco, D. Rizopoulos, M. Kavousi.

First Health Sciences Research Day. Erasmus MC 2019 (Rotterdam Abril 11 2019)

Oral Presentation:

Impact of Cumulative Systolic Blood Pressure and Serious Adverse Events on Efficacy of Intensive Blood Pressure Treatment: A Randomized Clinical Trial

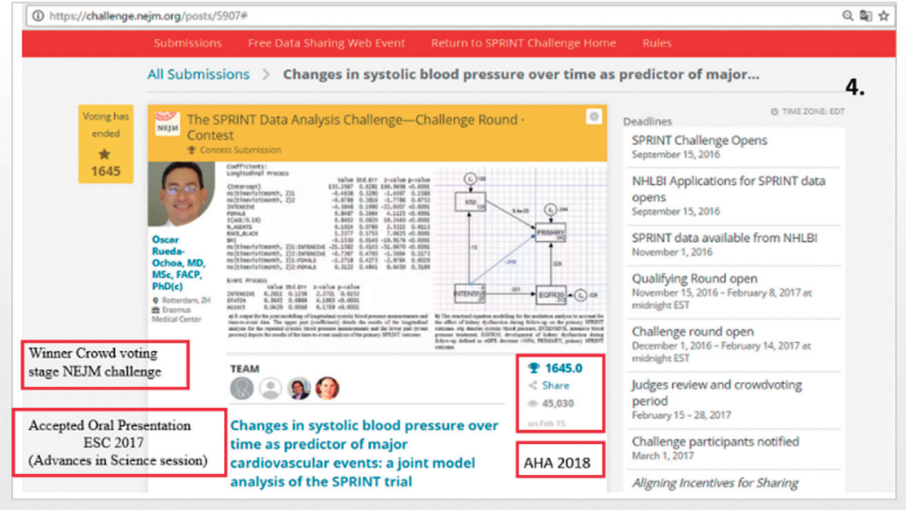
Oscar L Rueda-Ochoa, Lyda Z Rojas Sanchez, Shahzad Ahmad, Cornelia M van Duijn, M. Arfan Ikram, Jaap W Deckers, Oscar H Franco, Dimitris Rizopoulos, and Maryam Kavousi

2. International Research Competition February 2017

The SPRINT Data Analysis Challenge. The New England Journal of Medicine.

Changes in systolic blood pressure over time as predictor of major cardiovascular events: a joint model analysis of the SPRINT trial.

Winner crowd voting stage NEJM challenge



3. Graduation Master in Genetic Epidemiology NIHES (August 2017)

4. Contributions to Cardio-Metabolic research group and Rotterdam Study

- Update database abdominal aorta aneurysm cases in Rotterdam Study (working with Jelena Pavlovic).

- Calculation of ejection fraction in all RS cohort (working with Banasheh Arshi)

- Implementation of novel statistical methods: Generalized estimation equation models (GEE), marginal models, linear mixed models, Joint model analysis (Standard and cumulative), propensity score matching, competing risk analysis (Fine and Gray), Youden Index, structural equation modelling (SEM) and Mendelian randomization analysis.

- Advice to students: Marco Gelain, Lyda Rojas, Carolina Ochoa, Laura Pletsch, Hamid, Niels, Cindy, Janine, Elif, Banashah, Naushin, Ellie, Masoud, Fadila, Yuan, Lidia Bons.

- Collaborative work: Department of Cardiology, Vascular Surgery, Radiology, Neurology, Belgium Aalst group, Korean research group.

- 20/20 presentations (May 12 2017 and November 23 2018)

- All papers were present in the meeting of Cardio-metabolic research group (2016-2018).

5. Peer-Reviewer

Annals of Internal Medicine
ACP Journal club
British Medical Journal (BMJ)
Circulation
European Journal of Epidemiology
Nano-materials
International journal of nephrology and renovascular disease
Global Heart
Journal of American Heart Association (JAHA)
European Journal of Preventive Cardiology (EJPC)

Peer-reviewer annual Colombian health research competition – COLCIENCIAS 2018.
Peer-reviewer annual Colombian health research competition – COLCIENCIAS 2019.
Peer-reviewer annual Colombian health research competition – COLCIENCIAS 2020.

6. Participation in international epidemiologic networking

- Data methods Discussion forum (Frank Harrel`s website) https://discourse.datamethods.org/

- The Big Beat Challenge (The British Heart Foundation) https://bhf.flexigrant.com/

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I am grateful to Professor Oscar H. Franco for giving me the opportunity to join the cardiovascular research group that he directed in the Department of Epidemiology of Erasmus Medical Center, his permanent motivation and interest were vital to complete this thesis work. Also my feeling of gratitude to Professor Maryam Kavousi for the fruitful academic discussions, for believing in the challenges that this thesis posed, for her constant accompaniment, for the detailed review of all the articles that are part of the thesis, for always being available to answer my questions. My gratitude also to Professor Jaap W. Deckers, who always gave me a word of support, especially during the long and complex waiting periods for the publication of the articles. My gratitude also to Professor Dimitris Rizopoulos from whom I learned to understand the analysis of repeated measures and the Joint Model analysis, thank you very much for your total commitment to research projects shared especially for your hard work in the analysis of the SPRINT study.

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About the author

Oscar Leonel Rueda Ochoa was born in Barrancabermeja, Santander, Colombia (1966). He completed his undergraduate studies in medicine at the faculty of health of the Universidad Industrial de Santander UIS (1985-1991). During his undergraduate training, in his fourth academic semester, he was commissioned to manage the electrocardiography device of the hospital emergency department. With this experience, He made the first course of practical electrocardiography and created the research group on electrocardiography (1989-current), which in its beginnings was constituted by undergraduate students in medicine, nursing and physiotherapy. Recently graduated, he was named physician in the cardiovascular risk program at the cardiovascular Foundation of Colombia, the most important cardiovascular hospital in eastern Colombia and a teaching assistant in the department of basic sciences of Universidad Industrial de Santander (UIS), responsible for the cardiovascular physiology module. Subsequently, he completed a specialization in internal medicine (1995-1998) and was again linked to the cardiovascular foundation in the areas of coronary and postsurgical cardiovascular critical care (1998-2003), at the same time as he was appointed part-time professor in the department of Physiology of the UIS (1998 - current). He completed a master's degree in Clinical Epidemiology (2003) at the Pontificia Universidad Javeriana. He was named Fellowship of direct election of the American College of Physician (ACP) (2005) and later received the scholarship "International Fellowship Exchange Program" (2008) that allowed him to travel to make an academic internship at Pennsylvania University (UPENN). He received the "Excellence in Internal Medicine" award as the best internist doctor in Colombia (2009) by the Colombian Association of Internal Medicine (ACMI). In 2013, on the occasion of a medical research symposium, he met with Dr. Adriana Buitrago (graduated of MSc Epidemiology in Erasmus University) who talked him about Erasmus University academic program and contacted him to Professor Oscar H. Franco, who invited him to know the program. Oscar Leonel began his doctoral program at the Erasmus University Medical Center in July 2015, thanks to a scholarship awarded by COLCIENCIAS and a study commission from the Universidad Industrial de Santander UIS. During his doctoral stay he stood out for his participation in the "The SPRINT data analysis challenge" organized by the New England Journal of Medicine, obtaining the first place in the crowding voting phase with 1645 votes to his research proposal, which was read by 46205 people around the world. In August of 2017, He received the Master's degree in health sciences with emphasis in genetics epidemiology at Erasmus University. He will come back to Colombia to continue working at Universidad Industrial de Santander (UIS).

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Thesis prepositions (stellingen)

1. Factors related to the mother (maternal age and number of pregnancies), delivery (cesarean section) and newborn (weight at birth) can play a role in the dynamics changes over time in electrocardiographic parameters in the neonatal period. (This thesis)

2. Smoking among women and metabolic factors (diabetes mellitus and body mass index) among men showed larger deleterious associations with longitudinal changes in left ventricular diastolic function parameters over time. (This thesis)

3. The initial beneficial impact of intensive hypertension treatment might be offset by cumulative systolic blood pressure, higher systolic blood pressure variability and development of serious adverse events during follow-up. (This thesis)

4. In endovascular abdominal aortic repair (EVAR), patient selection and meticulous peri-operative care is key to reduce post-operatory complications and increase survival (This thesis)

5. Descending aortic diameters emerge as a new risk marker strongly associated with stroke, heart failure and cardiovascular mortality in women. (This thesis).

6. The availability of more sophisticated methods for analyzing repeated measures data, as linear mixed models, is an important step in advancing our understanding of the factors associated with changes in biological parameters over time.

7. Overall effect of a medical intervention, including both the direct and indirect effects, could be better evaluated under a joint model analysis approach.

8. In observational studies and quasi-experimental studies, selection of controls under a propensity score matching approach decreases the chance of bias and increases the power to detect significant differences between the groups under study.

9. The Science increases our knowledge and, hopefully, improves our lives by asking and answering relevant questions. Ann bless & Ed Hull. Reader-friendly biomedical articles. How to write them!. Third edition. 2012. Van Zuiden communications.

10. “Statistics, contrary to popular perception, isn’t really about facts; it’s about how we know or suspect or believe that something is a fact. It has more in common with philosophy (e.g. epistemology) than accounting. Statisticians are applied philosophers.” Stephen John Senn (@stephensenn)

11. Primum non nocere

The development of health sciences research goes hand in hand with advances in the design of epidemiological studies as well as the mathematical tools for the analysis of biomedical information. Novel statistical analysis tools such as the Cox proportional hazard model and its extensions, linear models for repeated measurements of continuous data such as linear marginal and mixed-effects model and generalized estimation equations (GEE), joint models for longitudinal and to time-to-event data, propensity score matching, and advances in the area of genetic epidemiology such as the development of genetic risk scores and Mendelian randomization analysis, have narrowed the gap between association studies with those aiming to establish causality. This thesis applied all of these novel statistical tools to contribute to answering research questions regarding dynamic changes in the structure and function of the cardiovascular system.