A multi-site approach to vascular age

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Abstract

Introduction: Cardiovascular disease is a major burden on healthcare systems worldwide. Vascular age, an estimate of an individual's cardiovascular age from a medical perspective, has shown promise in improving cardiovascular risk management.
Problem: Vascular age models currently only consider the calcification status of the coronary arteries. However, carotid artery calcification predicts stroke better than coronary calcification does. The role of calcification of the carotid arteries and other arteries on vascular age estimation has yet to be studied.
Aims: The aim is to establish the role of multi-site arterial calcifications in vascular age.
Design: Cross-sectional cohort study
Methods: Calcification volumes of the coronary arteries, aortic arch, vertebrobasilar arteries, the extracranial, and the intracranial carotid arteries were collected for 1721 community-dwelling elderly (average age 68.2 years). After a statistical analysis of the data, multiple linear support vector regressors (lSVR) were trained on the available calcification data (features) to estimate chronological age (label). The lSVR-estimated age was used as a proxy for vascular age.
Result: The multi-site lSVR had a mean absolute error (MAE) of 4.0 years on the test dataset. Only the aortic arch lSVR (MAE 4.03 years) did not perform worse than the multi-site lSVR on the test dataset (p=0.316), however, the multi-site model did perform better on the training dataset (p=0.021). The multi-site model outperformed all other single-site lSVRs on both the training and test dataset. Females showed a larger difference between vascular age and chronological age than males did (respectively 1.3 and 0.7 years lower vascular age, p=0.01).
Conclusion: The performance of vascular age models can be improved by including calcification volumes from multiple arterial sites.
Contribution: This is the first study that uses calcification volumes of multiple arterial sites to improve the estimation of vascular age.

Acronyms

AAC Aortic Arch Calcification. 21

BA Biological Age. 12

CAC Coronary Artery Calcification. 7, 9, 13, 21, 58

CIMT Carotid Intima Media Thickness. 56, 57, 59

CVD Cardiovascular Disease. 7

CVRM Cardiovascular Risk Management. 7

ECAC Extracranial Carotid Artery Calcification. 21

ICAC Intracranial Carotid Artery Calcification. 21

lSVR linear Support Vector Regressor. 27, 41

MAE Mean Absolute Error. 26, 27

ML Machine Learning. 16

MSCT Multi-slice CT. 21

PIT Pathological Intimal Thickening. 14

PWV Pulse Wave Velocity. 58

VBAC Vertebrobasilar Artery Calcification. 21

Glossary

asymptomatic Not showing any signs or symptoms of disease. 7

clinically relevant Term used to denote that something has sufficient value such that it could impact how medical professionals 'in their clinic' practice medicine. 12

endogenous As produced by the body itself. 54

Estradiol Estradiol is a steroidal estrogen hormone and the major female sex hormone. 54

myocardial infarction heart attack. 14

p-value Assuming that a specified null-hypothesis holds, the p-value is the probability of obtaining a result at least as extreme as the results that were observed according to a statistical test. 39

pathological Symptom or sign indicative of disease. 14

thrombus blood clot. 14

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Part I

Prologue

Chapter 1

Introduction

1.1 Problem Statement

Cardiovascular Disease (CVD) is a global burden on health care systems. CVD is the cause of one in three deaths worldwide. Ischemic heart disease, which includes heart attack, is the most common cause of cardiovascular death. Stroke, which includes both cerebral infarction and cerebral hemorrhage, is the second most common cause. Together they are responsible for 85% of CVD deaths worldwide [1].

Prevention of cardiovascular incidents in asymptomatic individuals is based on predicting their cardiovascular risk [2]. Traditionally risk assessment has been performed by calculating a risk score based on known cardiovascular risk factors. The most widely used risk score is the Framingham risk score [3].

Risk scores perform modestly because there remains a considerable overlap between the risk estimation of individuals that develop cardiovascular disease and those who do not [4, 5]. Improving risk prediction leads to an improved classification of high and low-risk individuals. In turn, this improves preventative care and decreases potential over- and under-treatment [6]. To improve cardiovascular care better risk estimation is needed.

Adding information about atherosclerosis to risk estimation models is a promising approach because atherosclerosis underlies the pathogenesis of cardiovascular disease [7]. As atherosclerosis progresses, atherosclerotic plaques can calcify. These calcifications can be measured using computed tomography (CT) scanning.

Vascular age, derived from Coronary Artery Calcification (CAC) as measured by CT, has been suggested to improve Cardiovascular Risk Management (CVRM). Using a CAC-based vascular age in risk scores has been shown to lead to clinically relevant improvements in risk calculations [8]. CAC is suitable to improve heart-related cardiovascular disease

risk estimation. CAC can be used in practice to improve whenever the measurement is available [9].

Unfortunately, the usage of CAC in cardiovascular risk prediction has its limitations. In a large population study, after adjusting for carotid artery calcification, CAC was not significantly related to the presence of stroke [10]. Thus CAC might not be a suitable metric to predict stroke. Therefore, multi-site calcification assessment might lead to better cardiovascular risk prediction.

CAC is currently the only arterial calcification metric that has been used to calculate vascular age [8]. In this paper, we address this gap by exploring a more comprehensive approach to vascular age by investigating the calcification status of multiple arteries.

1.1.1 Research Questions

This research consists of two parts. The first part is a data-analysis, in which we will investigate the relations between age, arterial calcification volumes, and sex in the collected data. The second part leverages what was learned from the first part. In the second part, we will build vascular age models. These models were investigated to elucidate their performance and behavior. This separation between those two parts of this research is reflected in the research questions. The research questions of the data-analysis part will start with the digit "1". For the vascular age research part, the starting digit is "2".

Research Questions — Data Analysis Part

If the calcification volumes of different arteries are (close to) perfectly correlated, we can (close to) perfectly estimate the volumes in both arteries by measuring just one artery. Therefore, measuring different sites is only useful when the correlation is not too strong. Note that a correlation can be considered too strong if the additive information of another site is not (medically) relevant. Here we will arbitrarily use the term "strong relation" only when the correlation between two sites is $\rho > 0.75$. Finding a sufficiently strong relationship would be useful because then we could just measure the most medically amenable artery. If the correlation is not high, this could mean that both sites might individually provide relevant information. Therefore we aim to answer:

> *Research Question 1a*
> What is the correlation amongst calcification volumes across spatially-distinct vascular sites?

We will build models that use calcification volumes to estimate someone's age. Note that this age estimate will be derived from the arterial calcification volumes and will be called vascular age henceforth. Before we can do this, we want to know what the relations between age and arterial calcification are. Therefore we will first the following:

Research Question 1b
What is the correlation between age and arterial calcification volumes?

Males and females are known to differ in the mortality [11], onset [12], and presentation [13] of cardiovascular disease. Females are also known to have lower levels of Coronary Artery Calcification (CAC). Therefore, we would like to perform a separate analysis of a female and male dataset to answer:

Research Question 1c
What sex differences exist in the calcification status of the different sites?

Research Questions — Vascular Age Part

Currently, only the importance of CAC for vascular age is known. Although CAC is strongly related to ischemic heart disease, it is less useful to predict stroke [10]. Carotid artery calcifications are more strongly related to stroke [10]. However, the role of the carotid calcifications and other arteries in vascular age is unknown.

Adding calcification volumes of other sites might make vascular age estimations more accurate. We aim to address the limitation of current vascular age models by exploring the role of calcifications in multiple arteries. Therefore we want to answer the following research question:

Research Question 2a
Do multi-site calcification models of vascular age contribute to a more accurate vascular age estimate compared to single-site (in particular CAC-only) models?

It is unwise to CT-scan larger volumes of an individual's body if the information gained is not sufficiently relevant due to increased radiation exposure. One site might be more valuable to estimate vascular age than others. To learn which arteries calcification volumes are worthwhile to scan we aim to answer the following:

Research Question 2b
What is the performance of single site models for the coronary arteries, the aortic arch, the vertebrobasilar artery, the extracranial carotid, and the intracranial carotid arteries, in the estimation of vascular age?

Females and males differ with regard to cardiovascular disease. For example, females have higher mortality than males even at the same level of CAC [14]. Since this is the first time a multi-arterial approach to vascular age is taken, we want to elucidate any differences in

sex-specific models. We aim to answer the following:

Research Question 2c
What is the impact of sex on the estimation of vascular age?

1.1.2 Research Methods

To answer the research questions, we first perform a data analysis on a large calcification dataset provided by the Department of Epidemiology of the ErasmusMC. Here we explore the relations between calcifications and chronological age on a per-vessel basis.

Using statistical and machine learning models, we'll calculate vascular age based on calcification information. We'll build models with the following configurations:

- A model that takes calcification volumes for all sites into account
- A model for when just a single site's calcification volume is available. (We will build one model for each site).
- One model that is specifically tailored for females and one for males.

We validate the models' performance by comparing them to each other and to a baseline model.

1.2 Contributions

This research makes the following contributions:

1. This is the first paper that studies the relation between vascular age and arterial calcifications besides the coronary arteries.
2. This paper explores if multi-site calcification assessment could be a valuable addition to vascular age estimation which, in turn, might be used to improve cardiovascular risk assessment.
3. This paper explores the role of sex in the relation between calcification volumes and vascular age.
4. This is the first paper that explores the use of machine learning to improve the estimation of vascular age.

1.3 Outline

In Chapter 2 we describe the background of this thesis. Part II describes the methods that were used to collect the data, to perform the initial data analysis, and to build and

validate the vascular age models. Chapter 6 describes the result of the data analysis. The results of the vascular age models can be found in Chapter 7. In Chapter 8 the results of the data analysis and the result of the vascular age models are discussed. Chapter 9, contains the work related to this thesis. Finally, we present the concluding remarks in Chapter 10.

Chapter 2

Background

This chapter will present the necessary background information for this thesis. First, we briefly introduce the concept of biological age. We follow this up with the basic medical knowledge required to understand the vascular aging process. Finally, we briefly describe the use of machine learning to estimate an individual's vascular age.

2.1 Biological Age

Most developed countries accept the chronological age of 65 years as a threshold for an older person [15]. However, the rate of age-related decline varies [16]. For example, at one end of the spectrum, we have the 60-year-old with a large medical history and a substantial need for care to accomplish activities of daily living. At the other end, we have an active and self-sufficient 80-year-old, seemingly resilient to age-related disease. Although the 80 year-old in this example is chronologically older, biologically the 80-year-old might be as old, or even younger, than the 60-year-old. The concept of Biological Age (BA) has been introduced to quantify this seemingly different rate at which individuals age [17]. BA has been used to improve the risk prediction for mortality and late-life morbidity [17–20].

Although a well-established concept, BA lacks a generally accepted definition. BA is typically described as the "real aging state" of an organism. (Also see Section 2.1.1.) In general, BA aims to better describe "true life expectancy" or "healthy life expectancy" than chronological age does [21]. Individuals age "successfully" by remaining in good physical fitness and thus have a lower BA than their chronological age.

2.1.1 Varying Aging Rate of Different Organ Systems

It is not straightforward to explain BA using a single biomarker because tissues age at different rates [22]. A few examples of some well-researched and clinically relevant biomarkers

of aging [22] are listed in Table 2.1.

Table 2.1: Markers of Biological Aging

Biomarkers of Aging	Organ System
Systolic blood pressure increases with age	Cardiovascular system
Grip strength decreases with age	Musculoskeletal system
Forced expiratory volume decreases with age	Lung system
Cholesterol increases with age	Cardiovascular system, Metabolic System
Average glucose blood level increases with age	Metabolic System
Cognitive functioning decrease with age	Cognitive system

The variability of the aging of tissues is also reflected by the type of diseases that individuals develop. For example, one might develop cardiovascular problems relatively early in life but still have outstanding joint health at a later age. To research these different rates of aging it makes sense to have different types of biological ages for different organ-systems. These different types of biological ages can be used by clinicians to organize the prevention of the diseases that their patients are most susceptible to.

The concept of vascular age has been introduced to better capture the rate of cardiovascular aging than chronological age does. Vascular age has been shown to lead to more accurate risk stratification of patients [23], Additionally, the concept of vascular age is also used as a communication tool by clinicians. Vascular age improves patients' understanding of their cardiovascular risk and it has been shown to improve adherence to lifestyle changes [8].

Vascular age derived from Coronary Artery Calcification (CAC) measurements improves ischemic heart disease risk estimation [8, 23], So far, only the calcification status of the coronary arteries has been used to estimate vascular age. It is known that individuals develop atherosclerotic calcifications at variable rates in different arteries [24]. Therefore it is possible for individuals to have no sign of coronary artery calcification but have above average amounts of calcification in their carotid arteries. The different calcification sites have also been shown to relate to different types of cardiovascular disease. Whereas CAC might be strongly related to ischemic heart disease, carotid artery calcifications are more strongly related to stroke [10].

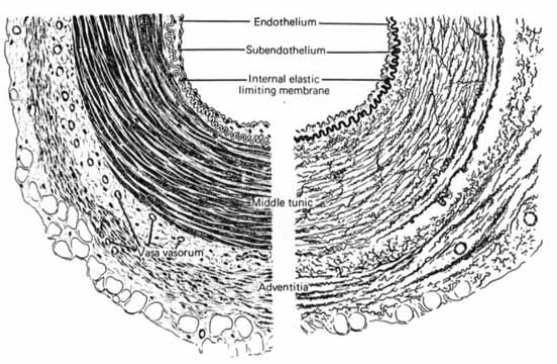
2.2 Pathophysiology of Arteriosclerosis

Atherosclerosis is the number one cause of mortality and disability in the developed world [25, p2]. Atherosclerosis is the buildup of plaques inside the arteries.

The process of atherosclerosis is a lifelong progression that starts early in life. Autopsies of trauma patients aged 2–15 years show that 50% already developed early stages of atherosclerosis [26]. Atherosclerosis is classified according to this progression [27].

The earliest pathological atherosclerotic vascular changes are microscopic. Lipoproteins, especially low-density lipoproteins, start to accumulate in the middle layer of the arteries called the tunica media. The tunica media can be found labeled "middle tunic" in Figure 2.1. This thickens the tunica media and is called Pathological Intimal Thickening (PIT) and is mainly caused by the accumulation of lipids [27].

Figure 2.1: Arterial Layers



Later in this process, macrophages — a type of white blood cell — accumulate in the lesions of PIT. These macrophages internalize the accumulated lipoproteins and form cholesterol [28]. PIT-lesions with macrophages — at this stage called foam cells — are considered to be more advanced plaques [27].

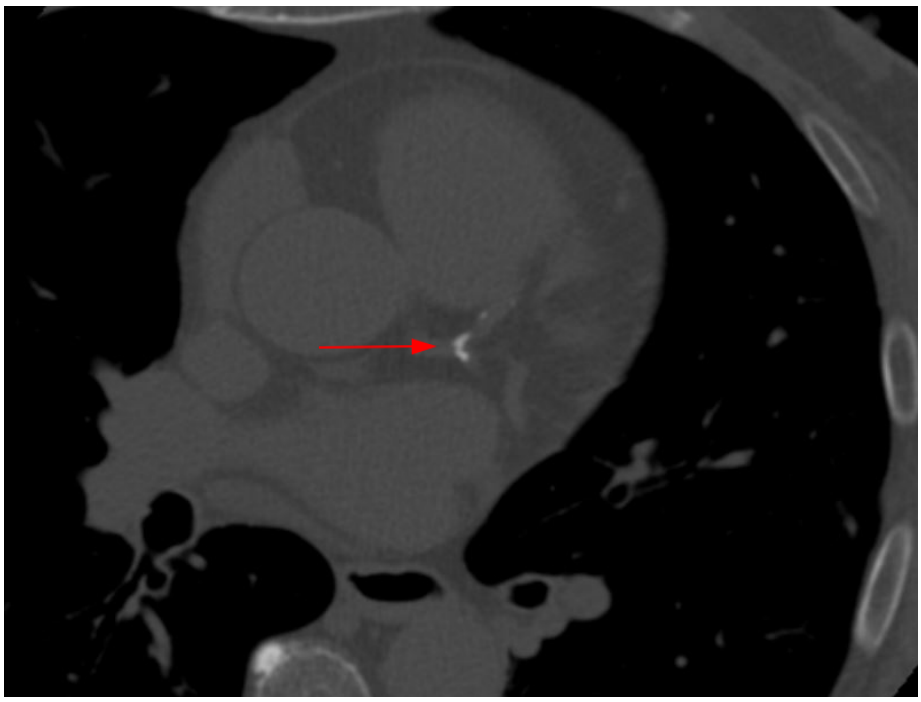
As a lesion starts to grow, it becomes harder to properly oxygenate the core. Eventually, the foam cells in the core become apoptotic. At this stage, a lesion is called a fibroatheroma. The necrotic core is covered by a fibrous cap that provides varying levels of integrity. As macrophages die, they release, among other things, cholesterol and calcium. And, over time, cholesterol and calcium crystallize [27].

At this stage, the fibroatheroma can rupture especially if it has a weak and thin fibrous cap. The fibroatheroma may also erode which can cause a thrombus to form in the tunica intima. In the case of the coronary arteries, a thrombus may block the blood flow to the heart muscle. This is a common cause of myocardial infarction [27].

Atherosclerosis is a systemic disease that is known to occur in all arteries in some form or another. However, both the rate of progression and the composition of atherosclerotic lesions are variable between individuals and between arteries within a single individual [24, 27].

Atherosclerotic plaques with sufficient calcium deposits can be visualized using a CT scan. Calcium is more radiodense than the surrounding vascular tissues and thus are brighter on CT scans. An example can be seen in Figure 2.2. This figure shows calcium deposits located in a coronary artery.

Figure 2.2: CT-scan showing coronary artery calcification



2.3 Cardiovascular Risk Estimation

One of the earliest studies on cardiovascular risk factors — the Framingham Heart Studies — started in 1948 [29]. After decades of research, this led to one of the best-known risk profiles, the "Framingham Risk Score", published by Wilson et. al. in 1998 [30]. Wilson established a scoring system with risk-charts that doctors could use in clinical practice [30]. Using the risk factors mentioned in Table 2.2 doctors could establish a risk score. This score could be used to derive the 10-year risk of developing coronary heart diseases. An example of such a risk table can be found in this publication [30].

Table 2.2: Traditional Cardiovascular Risk Factors

Risk Factor
High Chronological Age
Increased LDL-cholesterol and/or increased total cholesterol
Decreased HDL-cholesterol
Increased blood pressure
Presence of diabetes
Presence of smoking behavior

Traditional Risk Factors For Cardiovascular Disease as published by [30]

2.4 Machine Learning

The term Machine Learning (ML) was first coined by Arthur Samuel in 1959, when he was researching computer learning for IBM, by creating a program that could play checkers [31]. ML is related to statistics, computer science, and mathematical optimization and it is a subfield of artificial intelligence. The field of ML is focused on creating self-optimizing algorithms that automatically detect patterns in data without being explicitly programmed to follow certain rules. Such an ML-algorithm can then be used to make predictions and decisions.

2.4.1 Machine Learning Basics

In supervised ML an algorithm is "trained" by data. The input for the training process is the data (which describe certain *features* of the object that the ML algorithm is trying to "learn") and the correct answer (also known as the *label*). The algorithm then determines what the important features of the data are to predict the label. The aim is to train this algorithm to generalize to data from the general population. As a result, we can use the algorithm to predict the correct label even if it hasn't seen an individual's data before. In practice, this general population is represented by a *test dataset*. This test dataset is created by holding out a part of the entire dataset. The ML-model never gets to see the data in the test dataset except at the very end, when the model's performance is assessed. The remaining data is used to train the model and thus is known as the *training dataset*.

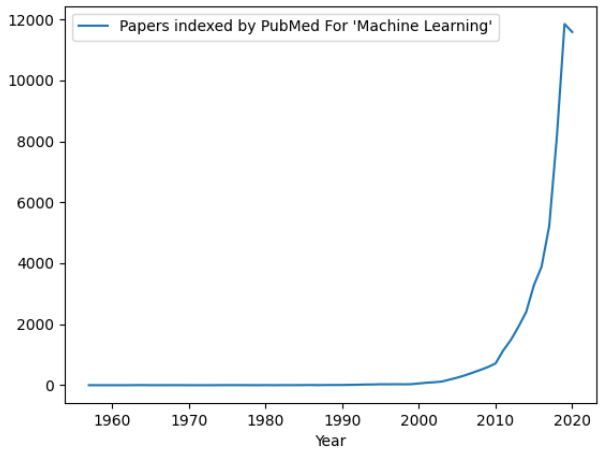
2.4.2 Machine Learning Usages

ML is being used to perform every increasingly difficult tasks. ML is used for online video recommendations [32], spam filtering [33], detection of fraudulent financial behavior of individuals [34] and corporations [35], chatbots [36, 37], voice recognition [38], automatic translation [39], and it has been used to defeat elite players in games such as chess and go [40].

Machine Learning Usages in Medical Research

As ML is proving itself more useful over time, it is not unexpected to see an increase of interest by medical researchers. Before 2003, fewer than 100 articles were indexed every year by PubMed. This number grew to over 1000 papers in 2010, and with 2020 still not over (November 2020 as of this writing), over 11.000 ML articles already have been indexed by PubMed this year. The trend of papers indexed by PubMed when searching for "Machine Learning" is shown in Figure 2.3

Figure 2.3: Trend on the number of papers on Machine Learning



The number of papers indexed by PubMed per year when searching for "Machine Learning". Before 1990 fewer than 10 papers were indexed every year. This number has grown to over 11.000 per year since 2019.

As a concrete example of ML, in pathology, ML-algorithms have been used to distinguish between cancerous and non-cancerous histological slides. In this case, the slides are either labeled as benign or malignant. Based on its performance, the ML-algorithm adjusts its internal parameters to get better at classifying a histological slide image [41]. These types of problems, where an ML-algorithm tries to classify data into two groups, are known as *binary classification problems*.

In radiology, ML is being researched to automatically diagnose patients. Recently, Li et. al. used ML to detect COVID-19, pulmonary embolism, and community-acquired pneumonia based on lung CT-scans. This type of problem, where an ML-algorithm tries to classify data into more than two groups, is known as a *multi-class classification problem*.

In health care planning, ML has been used to predict the costs of stay of patients [42]. In laboratory medicine, ML has been employed to predict test results, such as ferritin levels,

using the result from other tests [43]. These types of problems, where an ML-algorithms tries to estimate a continuous value, are known as *regression problems*.

Note that a comprehensive explanation of ML is out of the scope for this section. Instead, we refer readers interested in the use of ML in epidemiology and radiology to [44] and [45].

2.4.3 Machine Learning and Biological Age Estimation

To estimate an individual's age, ML is a popular approach [46, 47]. One example where ML has frequently been used is in the estimation of brain age. Here, a typical approach includes training an ML-model to estimate an individual's brain age using imaging data [48]. Another example is the usage of ML to derive a BA from electronic medical records [49].

Although many different approaches exist to estimate BA, ML models try to minimize the difference between the predicted BA and the chronological age of an individual [47]. The difference between the estimated (i.e. biological) age and the chronological age is known as the age gap. This age gap (i.e. the error of the model) is associated with the prevalence of disease. In formulaic terms:

$$\text{age gap} = \text{biological age} - \text{chronological age}$$

The age gap is of interest in risk estimation. Reducing the error of an ML model that estimates age to zero (i.e. the model can estimate chronological age without error) has been shown to reduce the ability of that model to estimate risk well [47]. Intuitively this makes sense because such a model says little about biological age and could be replaced by chronological age.

Part II

Methods

Chapter 3

Methods of Data Acquirement

In this chapter, we discuss how the data was acquired by the people of the Erasmus MC. Chapter 4 describes how the data analysis was performed. In Chapter 5 we describe which models were built and how their performance and behavior were assessed.

3.1 Participants

This study is linked to the Rotterdam study. Starting in 1990, inhabitants of Ommoord, a suburb of Rotterdam, were invited and agreed upon participating in this long-term population-based study. Detailed information regarding the Rotterdam study can be found elsewhere [50].

All participants that came to the research center between June 2003 and February 2006 for their regular follow-up were invited to participate in an additional CT-study. A total of 2524 (76%) of them underwent CT-scanning and 1775 participants also underwent Magnetic Resonance Imaging (MRI). The rationale for exclusively including participants that underwent MRI-scanning can be found in Section 8.2. The data of these 1775 participants was used for this study.

The study was approved by both the Medical Ethics Committee and the Radiation Protection Unit of the Erasmus Medical Center. The full ethics statement provided by the Erasmus MC is shown in the gray box below.

Full Ethics Statement

The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR `www.trialregister.nl`) and into the WHO International Clinical Trials Registry Platform (ICTRP `www.who.int/ictrp/network/primary/en/`) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians.

3.2 Methods of the Data Retrieval

Participants underwent two non-contrast CT-scans during one scan session. One scan ranged from the aortic root up to the circle of Willis [51]. This scan was used in the analysis for the aortic arch, the carotid arteries, and the vertebrobasilar artery. The other scan was a cardiac scan to assess the coronary arteries. All scans were performed with a 16-slice or 64-slice Multi-slice CT scanner [10].

3.2.1 Analysis of the CT-scans

Five arterial sites were assessed for calcifications. (See Table 3.1). Arterial calcifications on CT-scanning were identified using a threshold of 130 Hounsfield units for all arteries [24, 51, 52]. If a site contained multiple calcifications, then the total calcification volume was calculated by summing the volumes for all found calcifications. All calcification volumes are expressed in mm^3. The assessment was performed by trained scan readers which were blinded to the clinical data. Inter- and intra-observer agreement on the scoring for all sites was excellent [53] (ranging from 0.96 to > 0.99) [10, 51].

Table 3.1: The investigated arterial sites

Sites	Abbreviation used for the calcifications at this site.
Coronary arteries	Coronary Artery Calcification (CAC)
Aortic arch	Aortic Arch Calcification (AAC)
Intracranial carotid artery	Intracranial Carotid Artery Calcification (ICAC)
Extracranial carotid artery	Extracranial Carotid Artery Calcification (ECAC)
Vertebrobasilar artery	Vertebrobasilar Artery Calcification (VBAC)

Coronary Arteries

To assess the coronary arteries, a cardiac scan was performed which started from the apex of the heart to the tracheal bifurcation. The assessment of CAC included the left main, left circumflex, left anterior descending, and the right coronary artery [24]. Dedicated software (Syngo Calcium Scoring, Siemens) was used to further improve the assessment of the calcifications [24]. The detailed CT-scan protocol can be found elsewhere [24].

Aortic Arch

For measuring the calcifications of the aortic arch, the root of the aortic arch was defined by the image where the ascending and descending aorta merge into the inner curvature of the aortic arch [24]. The calcifications assessed for the aortic arch started from the root of the aortic arch up to:

- the first 1 centimeter of the common carotid arteries
- the subclavian arteries directly beyond the origin of the vertebral arteries
- the vertebral arteries

Extracranial Carotid Arteries

Assessment for calcifications for the left and right external carotid arteries started 3cm proximal and ended 3cm distal of the carotid bifurcation [24],

Intracranial Carotid Arteries

Calcifications of the intracranial carotid artery were assessed starting at the horizontal segment of the petrous internal carotid artery and ended at the top of the internal carotid artery [52].

Vertebrobasilar Artery

Assessment of the vertebrobasilar arteries started from the location where the basilar and vertebral arteries meet and ended at the top of the basilar artery [51].

Chapter 4

Methods of Data Analysis

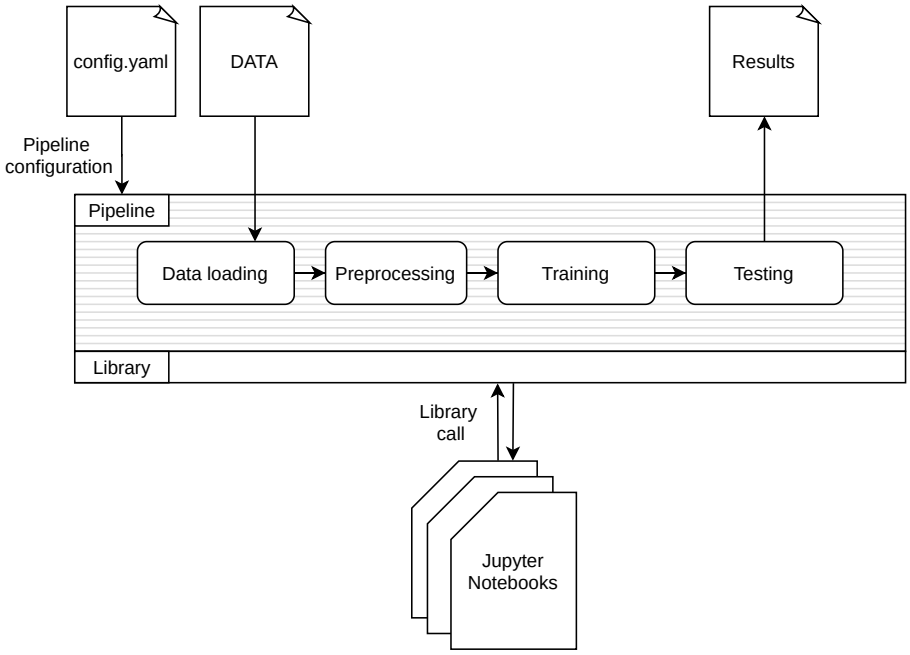
4.1 Developed Software

All the source code created for this thesis have been made publicly available and can be found at `https://github.com/melvio/vascular-age`. A configurable pipeline has been created that takes input data (e.g. calcification volumes) and produces output data (e.g. age estimations, time of the run, etcetera). This pipeline takes in configuration information, such as which type of scaling to use, and performs the entire analysis in a single run. This pipeline was written in an object-oriented manner and the individual objects can be reused which makes this project also suitable to be used as a library. For example, we used the objects of the pipeline as a library by importing the pipeline's object into Jupyter notebooks[1]. (See Figure 4.1).

All data analysis and model building and model validation were performed using the Python programming language version 3.8.2. A listing of the dependencies that were used for the analysis can be found in the appendix in Section A.1.

[1] `https://jupyter.org/`

Figure 4.1: Overview of the pipeline



4.2 Statistical Tests and Confidence Intervals

Statistical tests were considered to be significant at the $\alpha = 0.05$ level. All statistical tests were performed in a two-sided manner. Confidence intervals (CI) are 95% confidence intervals (95%-CI) ranging from the 2.5th percentile to 97.5th percentile unless noted otherwise.

4.3 Age Calculation

Age at the scan date is calculated up to one-day precision by calculating the difference between the birth date and scan date. The age at scan date will henceforth be referred to as the *chronological age*, or simply age, of the participant. Transformations from age in days to age in years are made using the following formula:

$$\text{age } \textit{(years)} = \frac{\text{age } \textit{(days)}}{365.25}$$

4.4 Analysis of Age, Calcification Volumes, and Sex

Spearman correlation coefficients were used to assess the correlation between the calcifications at different sites [54]. Spearman was also used to assess the correlation between calcifications and age. To compare the differences between females in males Mann-Whitney U-tests were performed.

Chapter 5

Methods of Vascular Age Modeling

5.1 Data Split

After dropping data entries that missed calcification volumes, the remaining data was randomly split into a training and test dataset. The split was performed using scikit-learn's `train_test_split` function [55] before selecting a model. Scikit-learn's default 75–25% train-test split was used, as this left us with sufficient data to train and validate the models. We used Mann-Whitney U-tests to see if the random split introduced differences between the training and test dataset.

5.2 Model Experimentation

We experimented with different preprocessing steps and ran different statistical and ML-models. A listing of models that we considered using can be found in Appendix A.2. During this experimentation, we only used data from the training dataset. We found that a power-transformation of the calcification volumes helped all models perform better compared to using min-max-scaling or using no scaling. No further preprocessing steps were applied.

To select a model to be used in the subsequent analysis we assessed their ability to estimate Mean Absolute Error (MAE). MAE is the average absolute difference between chronological age and the age estimation of a model. MAE is defined as follows, given that n is the

number of participants:

$$MAE = \frac{\sum_{i=1}^{n} |\text{estimated age} - \text{chronological age}|}{n}$$

To prevent overfitting, we ran these experimental analyses using K-fold cross-validation using different numbers for k. We ended up choosing a linear Support Vector Regressor (lSVR) since it performed well in the experimental analysis. Additionally, this model has the benefit of being easy to interpret.

5.3 Model Comparison

5.3.1 The Baseline Model

To assess models based on MAE, we need a baseline model that sets the lower bound for the performance. This baseline model tries to minimize MAE by discarding the features (here calcification volumes) and estimates a constant value for each individual in the dataset. The best performing constant estimator with regards to MAE is the median estimator (i.e. this is the constant estimator that best minimizes the MAE). The median estimator estimates the median age for each individual because this is the best way to minimize absolute error in the absence of calcification volume information. The performance of all models was compared with the performance of this median estimator. The median estimator is henceforth referred to as the baseline model. We consider a model to be outperforming the baseline model on a specified dataset if it has a significantly lower MAE according to a Wilcoxon signed-ranked test [56].

5.3.2 Model Performance on Test and Train Dataset

To check if a model overfitted on the training dataset, we tested if the MAE was significantly different between the two datasets using a Mann-Whitney U-test [57].

5.4 Multi-site and Single-site Models

For the multi-site model, we used an lSVR fitted to the power-transformed training dataset containing the calcification volumes for all five sites mentioned in Table 3.1. Five single-site lSVR were configured in the same manner as the multi-site lSVR however they were fitted to just a single site each. All single-site models that statistically outperformed the baseline model were compared against the multi-site model.

5.5 Sex Differences

5.5.1 Sex-Stratified Analysis

To analyze sex differences, we stratified the training dataset into a female and male training dataset and we did the same for the test dataset. For each sex, an lSVR was fitted to each training dataset. We compared the sex-specific lSVR with a baseline model and, again, we checked for differences in performance on the training and test dataset using a Mann-Whitney U-test. The feature coefficients of the lSVRs were used to assess the importance of calcifications at each site. The value of a feature coefficient of an lSVR is linearly related to its importance in the estimation of vascular age. This property allowed us to assess the relative importance of each site for determining vascular age for both sexes.

It is important to note that this analysis is not suitable to compare the vascular age of females and males. To compare the sexes directly we used a different approach.

5.5.2 Sex-Pooled Analysis

To compare the sexes directly, we used the outcomes from the multi-site analysis which is described in Subsection 5.4. Since the model was fitted on a mixed training group without having sex information, the vascular age estimates are directly comparable once we stratify the results into a female and male group. We used Mann-Whitney U-tests to show whether females had a significantly different vascular age and age-difference compared to males.

Part III

Results

Chapter 6

Results of Data Analysis

6.1 Study Participants Characteristics

Calcification volumes were available for all five sites for 1721 out of the 1775 participants (97.0%). The characteristics of the 1721 participants are described in Table 6.1.

Table 6.1: Participants Characteristics

Characteristic (n=1721)	Mean	St.dev	Range
Age (*years*)	68.22	5.88	59.02–91.86
CAC (mm^3)	232.81	500.40	0.00–6922.60
AAC (mm^3)	591.70	1054.94	0.00–10739.80
ECAC (mm^3)	86.80	175.89	0.00–2299.60
ICAC (mm^3)	98.95	170.15	0.00–1719.15
VBAC (mm^3)	3.57	18.81	0.00–441.99

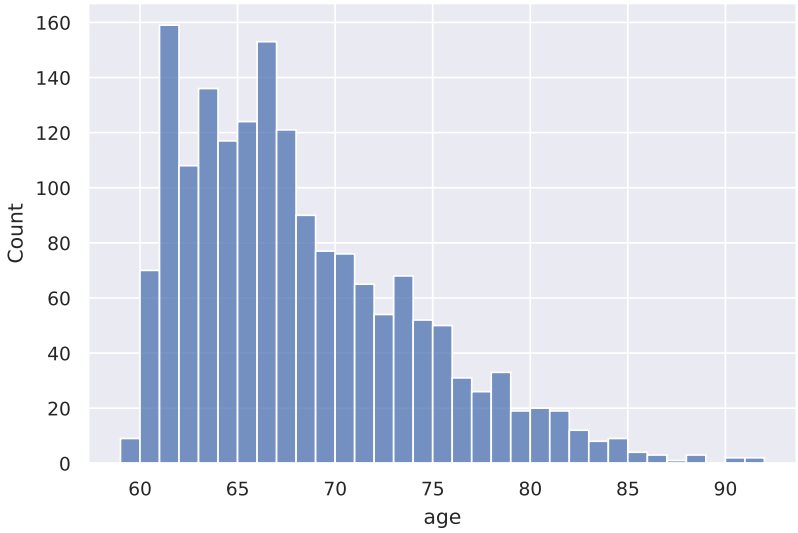
6.1.1 Chronological Age

The average age of the 1721 participants was 68.22 years. Age was distributed with a strong increase at 60 years due to the inclusion criteria and showed a gradual decline after 75, in line with life expectancy. As a result, 80% of the participants were within 61.6 (10th-percentile) and 76.6 years old (90th-percentile). The median age was 66.9. An age distribution plot is shown in Figure 6.1.

6.1.2 Arterial Calcifications

Calcification volumes were distributed according to an inverse exponential curve at all sites. The most frequently found calcification volume for every site was 0.0 mm^3 (no

Figure 6.1: Age distribution



Distribution plot showing the age of the 1721 participants. The bar widths span one year. The lowest bar starts at 59 years and the highest bar ends at 92 years.

calcifications). Having no calcifications was most common in the vertebrobasilar arteries (82.2% of individuals) and least common in the aortic arch (only 8.0% of individuals).

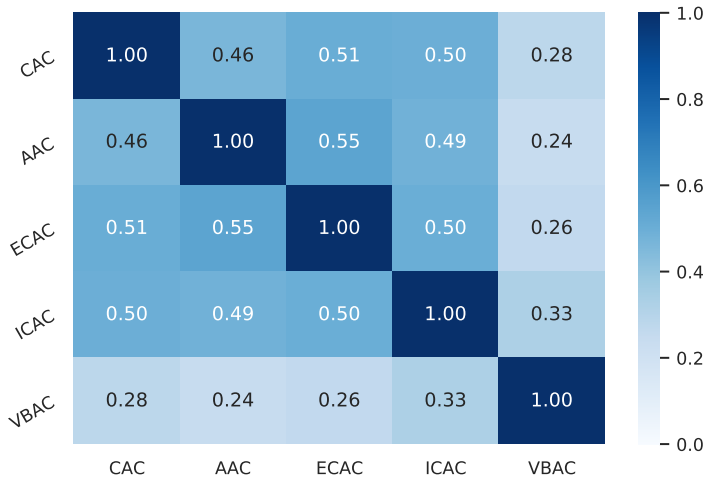
The Relations between the Calcification Volumes

Calcification volumes showed a low to moderate positive correlation with one another. VBAC showed a low correlation with CAC, AAC, and ECAC with a Spearman correlation coefficient of respectively 0.28, 0.24, and 0.26. CAC, AAC, ICAC, and ECAC were moderately correlated with a Spearman correlation coefficient ranging from 0.46 to 0.55. No sites were negatively correlated. A full correlation map that shows the relations between the different sites is shown in Figure 6.2.

The Relations between Age and the Calcification Volumes

The arterial calcification volumes varied widely at each site at every age. Linear and logarithmic calcification scatter plots with trend lines are shown in Figures 6.3, 6.4, 6.5, 6.6, and 6.7. In general, increased age was associated with increased calcifications for each site. This correlation was strongest for the aorta (r=0.34). The correlation between the calcification volumes for every site is shown in Table 6.2.

Figure 6.2: Correlation map between the calcification volumes at different sites.



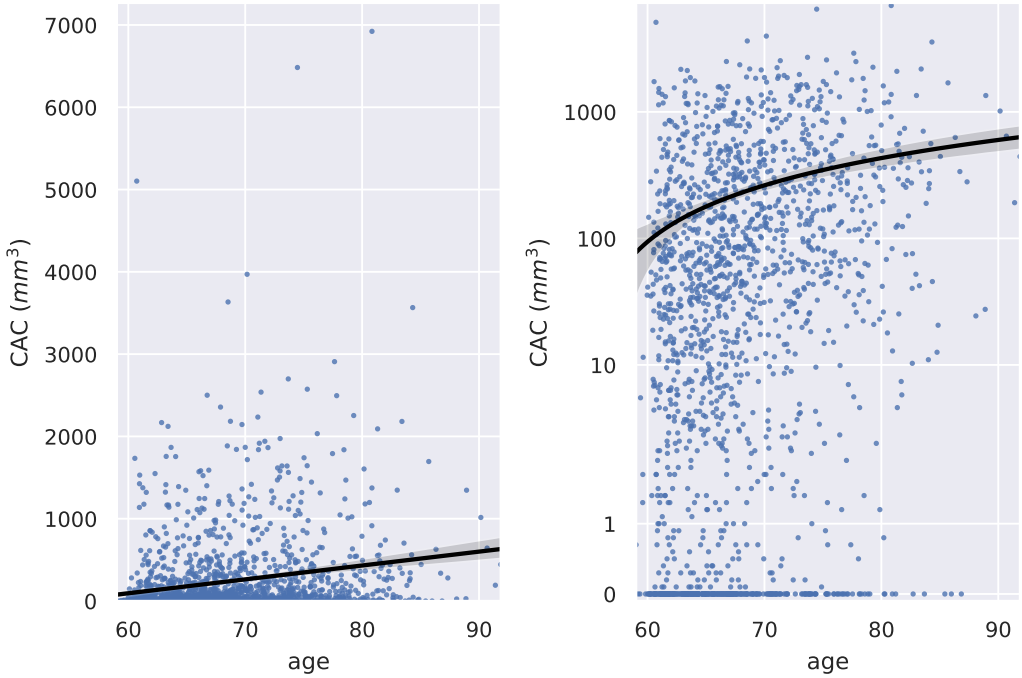
The mappings between the calcification volumes have been annotated with their Spearman correlation coefficients. A value of 0.0 indicates no correlation, a value of 1.0 indicates a one-to-one positive correlation, and -1.0 indicating complete inverse correlation. Most sites showed low to moderate correlation.

Table 6.2: Age Calcification Relations

Arterial Calcification	Correlation With Age
CAC	0.27
AAC	0.43
ECAC	0.27
ICAC	0.31
VBAC	0.19

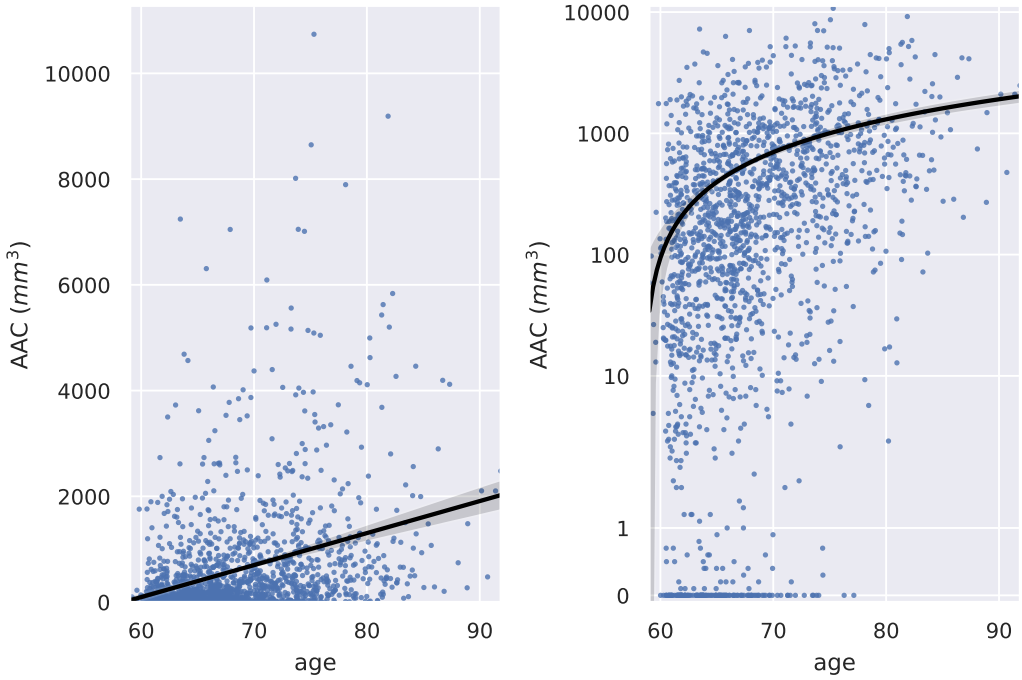
Spearman correlation coefficient between chronological age and the calcification volumes.

Figure 6.3: CAC Scatter Plot



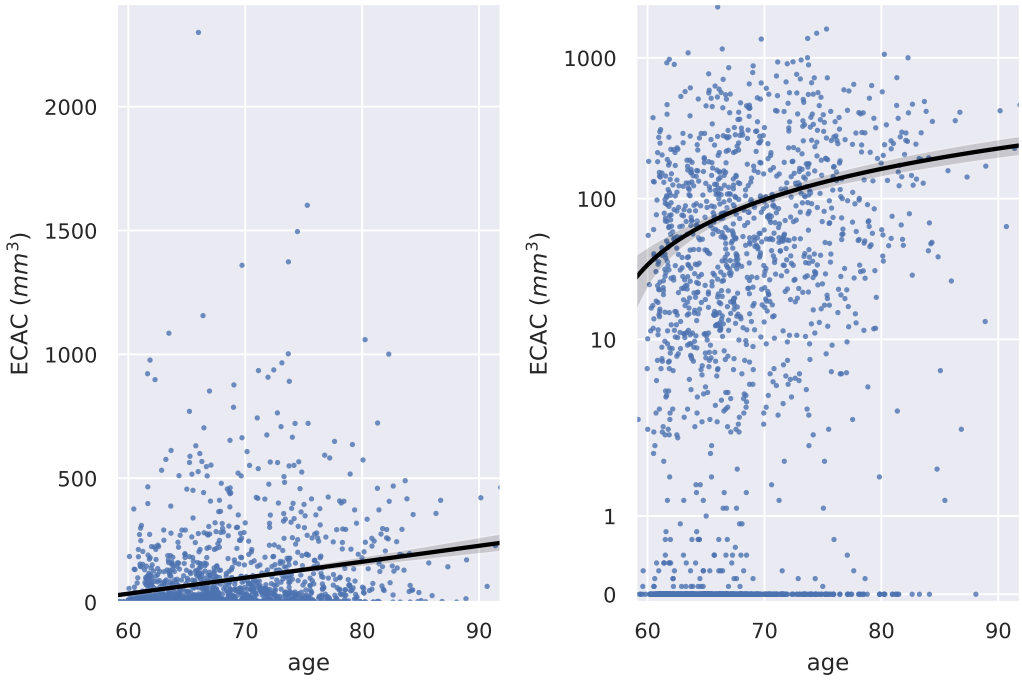
Scatter plots with age on the x-axis and CAC-volumes on the y-axis. The plot on the left uses a linear y-scale. The plot on the right uses a symmetric-log y-scale. The black line shows a regression line. The gray area around the black line shows the 95%-CI.

Figure 6.4: AAC Scatter Plot



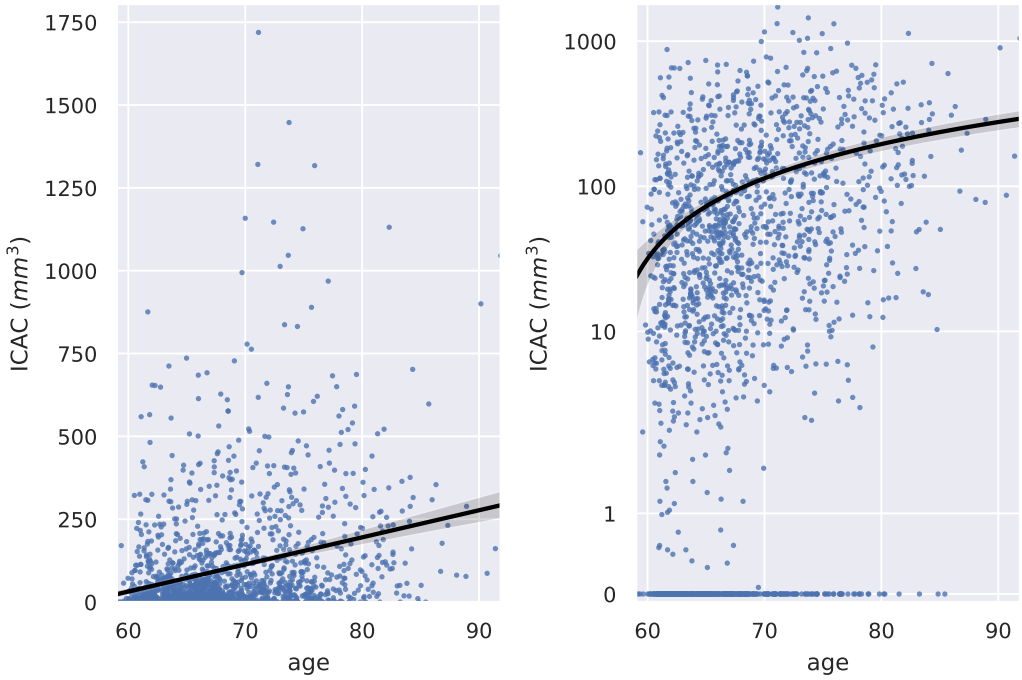
Scatter plots with age on the x-axis and AAC-volumes on the y-axis The black line shows a regression line. The gray area around the black line shows the 95%-CI.

Figure 6.5: ECAC Scatter Plot



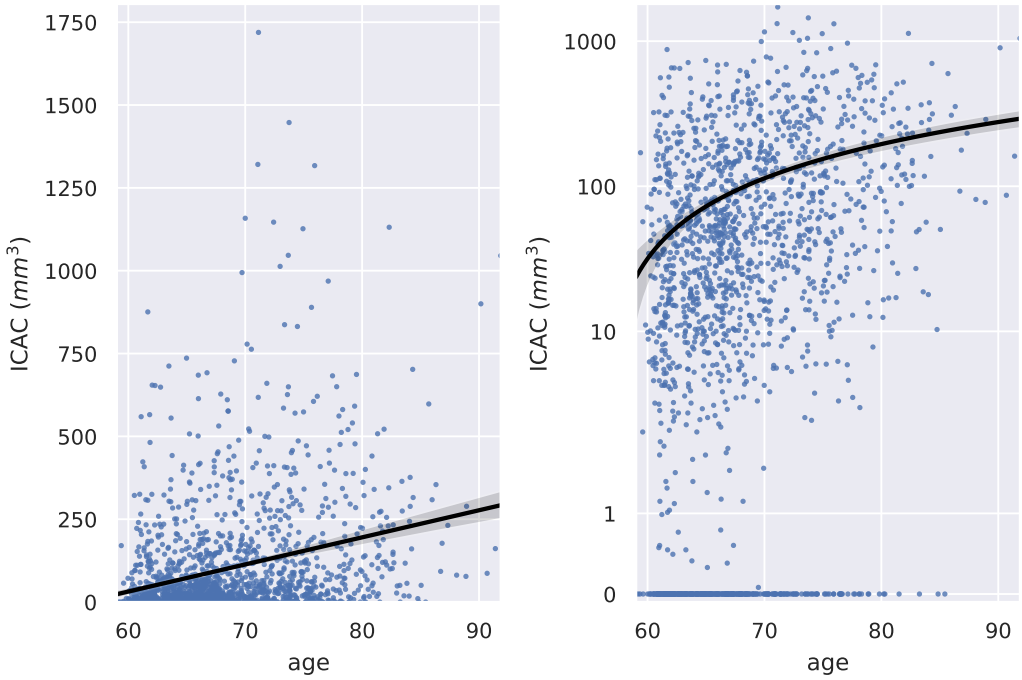
Scatter plots with age on the x-axis and ECAC-volumes on the y-axis. The black line shows a regression line. The gray area around the black line shows the 95%-CI.

Figure 6.6: ICAC Scatter Plot



Scatter plots with age on the x-axis and ICAC-volumes on the y-axis. The black line shows a regression line. The gray area around the black line shows the 95%-CI.

Figure 6.7: VBAC Scatter Plot



Scatter plots with age on the x-axis age and VBAC-volumes on the y-axis. The black line shows a regression line. The gray area around the black line shows the 95%-CI.

6.2 Comparative Sex Analysis

Out of the 1721 participants, there were more females (n=881) than males (n=840). This over-representation might be explained by women's higher life expectancy. Although this difference was not significantly different from a 50–50% binomial distribution (p=0.32–0.33).

Females were, on average, 68.2 years old. Only seven out of 881 females were completely calcification free. All calcification free females were younger than 70 years. Calcification information for females is shown in Table 6.3.

Table 6.3: Female Calcification Volumes

	CAC	AAC	ECAC	ICAC	VBAC
Mean	112.0	531.9	62.6	81.2	2.4
Minimal	0.0	0.0	0.0	0.0	0.0
25%	0.0	32.0	0.0	2.9	0.0
50%	10.0	175.7	9.1	27.2	0.0
75%	93.2	604.2	58.1	95.0	0.0
Maximum	2698.8	8014.9	1002.1	1131.1	186.3

This table shows the mean, minimal, 25% percentile, 50%, 75% percentile, and maximum calcification volumes (mm^3) for the females (n=881) for each site.

Males were, on average, 68.3 years old. Among males, there were also seven individuals without calcifications. The age of calcification free males ranged from 61.4 to 77.1. Calcification information for males is shown in Table 6.4.

Table 6.4: Male Calcification Volumes

	CAC	AAC	ECAC	ICAC	VBAC
Mean	359.5	654.4	112.2	117.6	4.8
Min	0.0	0.0	0.0	0.0	0.0
25%	14.4	37.9	0.5	6.3	0.0
50%	103.2	224.6	29.4	40.4	0.0
75%	416.1	737.0	128.2	140.9	0.0
Max	6922.6	10739.8	2299.6	1719.1	442.0

Male dataset (n=840) showing the mean, minimal, 25% percentile, 50%, 75% percentile, and maximum calcification volumes (mm^3) for each site.

Males had higher calcification volumes at all sites than females. This was especially pronounced in the coronary arteries. Males had in their coronary arteries, on average, over three times higher calcification volumes than females ($359.5mm^3$ respectively $112.0mm^3$). Females were also more often completely free of coronary artery calcifications (82 females

versus 13 males). The ratio of females to males with coronary calcification volumes over 1000 mm^3 was over 1:6.

In the aortic arch, males had 23% higher calcification volumes than females. However, the aortic arch was the only site where this difference did not reach statistical significance ($p = 0.051$). Males showed 79.4% higher ECAC and 44.9% higher ICAC volumes than females.

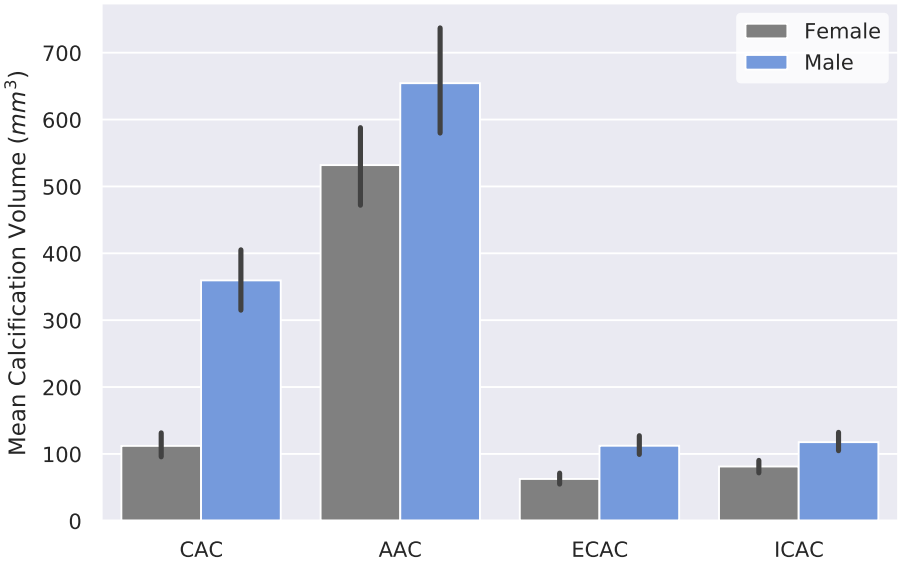
Calcification of the vertebrobasilar arteries was uncommon both in females and males. 88.0% of females and 80.5% of males were free of VBAC. VBAC volumes were less than 10 mm^3 for 831 out of 881 females and 759 out of 840 males. Males showed, on average, twice the amount of VBAC compared with females. A comparison of the levels of calcifications in females and males is shown in Table 6.5 and Figure 6.8.

Table 6.5: Age and calcification volumes per sex

Characteristics	Female	Male	Relative Difference*	p-value
Age ($years$)	68.2	68.3		0.410
CAC (mm^3)	112.0	359.5	+220%	> 0.001
AAC (mm^3)	531.9	654.4	+19%	0.051
ECAC (mm^3)	62.6	112.2	+111%	> 0.001
ICAC (mm^3)	81.2	117.6	+117%	> 0.001
VBAC (mm^3)	2.4	4.8	+100%	0.030

The characteristics of females and males (mean values). There was no statistical difference in age. The differences in the calcification volumes were significant for all sites but the aortic arch. The relative differences show how much higher the calcification volumes were in males. This has been calculated as follows $\frac{\text{volume female} - \text{volume male}}{\text{volume female}}$. The p-values have been calculated using a Mann-Whitney U-test.

Figure 6.8: Bar chart of calcification volumes



Calcification volumes for the coronary, aortic arch, intracranial and extracranial carotid arteries. Females are displayed with gray bars and males with blue bars. The 95%-CI are displayed with the black lines. Mean calcification volumes for the vertebrobasilar arteries (not shown) were 2.4 for females and 4.8 for males.

Chapter 7

Results of Vascular Age Modeling

7.1 Multi-site Analysis

The data was split into a training dataset (n=1290) and a (control) test dataset (n=431). There were no significant differences between both datasets. (See Table 7.1)

Table 7.1: Comparison of training and test dataset

Characteristic	Training dataset (n=1290) Mean [95%-CI]	Test dataset (n=431) Mean [95%-CI]
Age	68.3 [60.6–82.2]	67.9 [60.8–81.5]
CAC Volume	235.9 [0.0–1641.1]	223.5 [0.0–1353.8]
AAC Volume	603.3 [0.0–4040.0]	557.0 [0.0–3103.9]
ECAC Volume	87.5 [0.0–574.0]	84.5 [0.0–565.9]
ICAC Volume	101.1 [0.0–575.5]	92.6 [0.0–648.8]
VBAC Volume	3.9 [0.0–40.3]	2.5 [0.0–28.1]

There were no significant differences between the training and test dataset (all $p > 0.05$).

The linear Support Vector Regressor (lSVR) was able to calculate vascular age both on the training and test dataset. The MAE for the training dataset was 4.12 years. The lSVR generalized well to the test dataset and was slightly more accurate with an MAE of 4.00 years. The performance on the training and test dataset did not significantly differ ($p = 0.33$ Mann-Whitney U-test). The lSVR performed significantly better on the test dataset than the baseline model ($p > 0.001$ Wilcoxon signed-ranked test). The MAE of the baseline model was 4.63 years on the training dataset and 4.50 years on the test dataset.

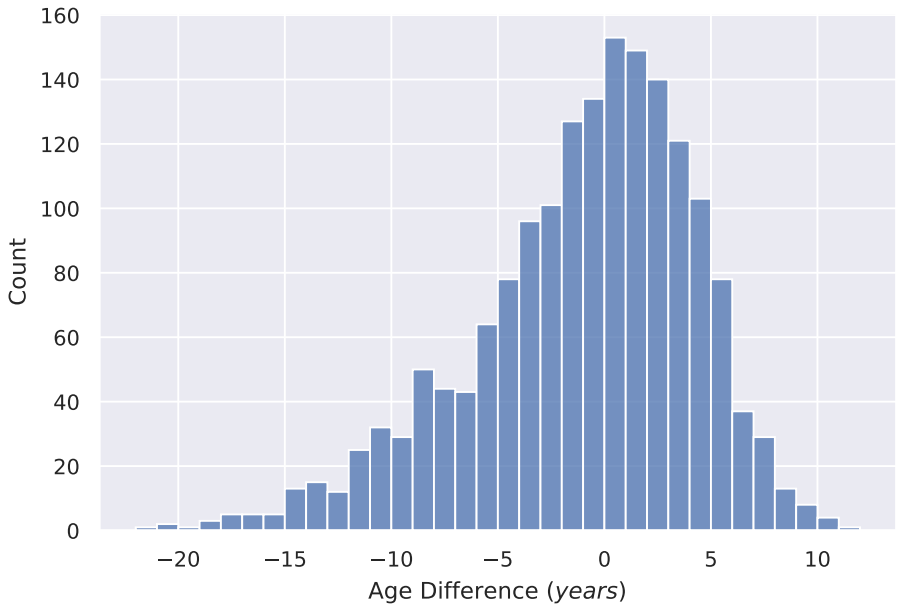
Individuals were estimated to be 1.0 years younger than their chronological age. This was -1.1 years for the training dataset and -0.7 years for the test dataset. (See Table 7.2) A histogram showing the difference between vascular age chronological age is shown in Figure 7.1. A scatter plot showing chronological age and vascular age is shown in Figure 7.2. The explained variance (R^2) on the training and test dataset were 0.156 and 0.197 respectively.

Table 7.2: Results on the training and test dataset

Characteristic	Training dataset (n=1290) Mean [95%-CI]	Test dataset (n=431) Mean [95%-CI]
Vascular Age	67.2 [62.6–72.8]	67.2 [62.8–72.4]
Age Difference	-1.1 [-13.2–7.3]	-0.7 [-12.6–7.1]

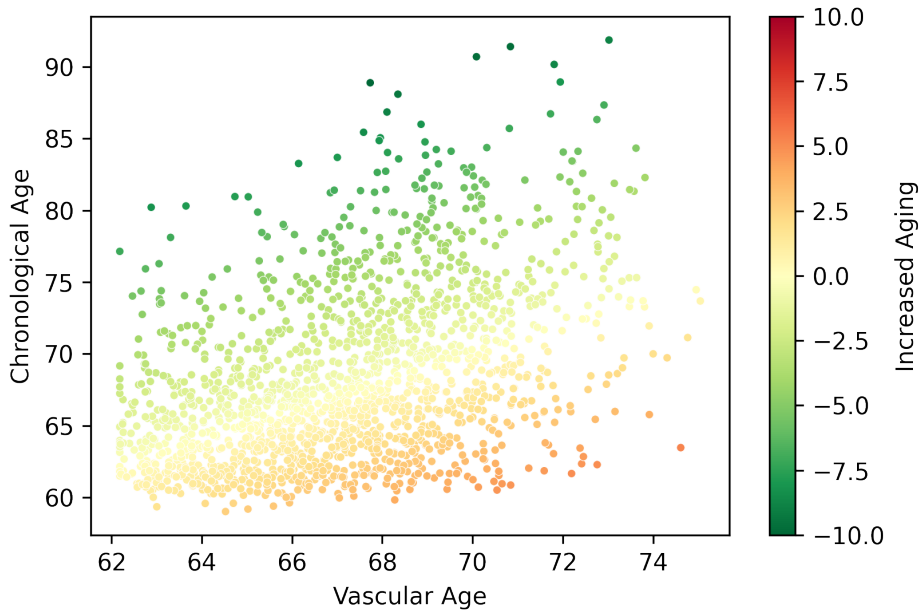
Table showing the estimated vascular age and age difference for the training and test datasets. A negative age difference indicates a lower vascular age than chronological age.

Figure 7.1: Histogram of the difference between chronological and vascular age



Age difference has been calculated as the difference between estimated (vascular) age and chronological age. A negative value indicates healthy aging. The bars have a width of one year. The leftmost bar starts at -22 and the rightmost bar ends at 12.

Figure 7.2: Scatter plot of vascular age and chronological age



The x-axis shows vascular age and the y-axis shows chronological age. Red indicates a higher vascular age than chronological age (i.e. unhealthy aging). Green indicates a lower vascular age than chronological age.

7.2 Single-site Analysis

The CAC-, AAC-, ICAC- and VBAC single-site lSVRs had a significantly lower MAE on both the training and test dataset compared to the baseline model (See Table 7.3). A Mann-Whitney U-test showed that no model performed significantly better on the training dataset than on the test dataset. The MAE, after fitting an lSVR on a single site calcification volume is shown in Table 7.4.

Table 7.3: Single-site models compared to the baseline model

Single Site Model	p-value (training dataset)	p-value (test dataset)
CAC	> 0.001	> 0.001
AAC	> 0.001	> 0.001
ECAC	> 0.001	0.197
ICAC	> 0.001	0.001
VBAC	0.034	0.046

The CAC, AAC, ICAC, and VBAC single-site models performed better (i.e. reduced MAE more) than the baseline model on both datasets. The p-values were calculated using the Wilcoxon signed-ranked test.

Table 7.4: MAE on the training and test dataset

Model Input	MAE on training dataset (years)	MAE on test dataset (years)
CAC	4.47	4.25
AAC	4.19	4.03
ECAC	4.42	4.39
ICAC	4.40	4.22
VBAC	4.54	4.38
*Baseline**	4.64	4.50

This table shows the MAE of chronological age after fitting an lSVR to the calcification volume at one single arterial site on the training dataset. The baseline model (i.e. the median estimator) is marked with a star (*). The lSVRs showed no significant difference in performance between the training and test dataset ($p > 0.05$).

7.2.1 Multi-site Analysis versus Single Site Analysis

For this analysis, we selected the four (AAC, CAC, ICIC, VBAC) single-site models that reached significantly better performance than the baseline model for a comparative analysis with the multi-site models. A Wilcoxon signed-ranked test based on the MAE showed that the multi-site lSVR outperformed the CAC-based lSVR both on the training ($p < 0.019$) and test dataset ($p < 0.001$). The multi-site lSVR was also more accurate than the ICAC-based lSVR on both the training and test datasets (respectively $p < 0.001$, $p = 0.004$). The

same was true for the VBAC-based lSVR where the multi-site model performed significantly better on both datasets (both $p < 0.001$).

The multi-site lSVR performed better on the training dataset than the AAC-based lSVR ($p = 0.021$). The multi-site lSVR was not significantly better than the AAC-based lSVR on the test dataset ($p = 0.316$). On the training and test dataset combined, the significance was $p = 0.014$ which shows that the multi-site model performed better than the CAC-based model when considering all the data.

7.3 Sex-Specific Analysis

The training and test dataset were further stratified based on sex. In the training dataset, 50.5% was female and in the test dataset, 53.1% was female. Sex did not significantly differ between the training and test dataset ($p = 0.352$ Mann-Whitney U-test).

7.3.1 Females

A Mann-Whitney U-test showed no statistically significant difference between the female training and test datasets. (see Table 7.5)

Table 7.5: Comparison of female training and test data

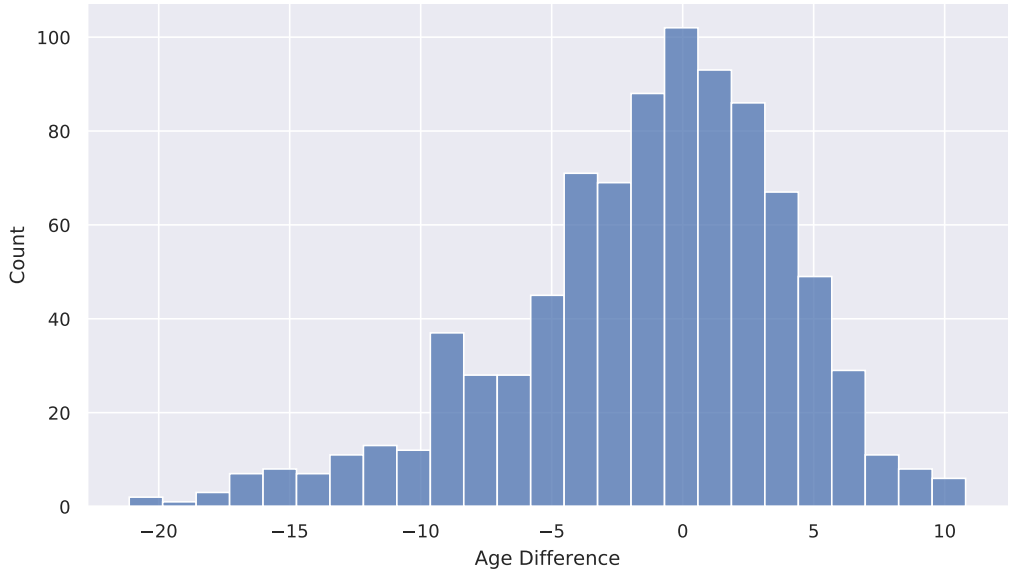
Characteristic (Females)	Training dataset (n=652) Mean [95%-CI]	Test dataset (n=229) Mean [95%-CI]	p-value
Age	68.3 [60.5–82.6]	67.7 [60.6–82.6]	0.122
CAC	110.1 [0.0–780.3]	117.4 [0.0–821.4]	0.992
AAC	553.7 [0.0–3715.0]	469.8 [0.0–2289.8]	0.730
ECAC	62.2 [0.0–450.1]	63.5 [0.0–528.3]	0.998
ICAC	82.1 [0.0–475.8]	78.4 [0.0–557.7]	0.374
VBAC	2.5 [0.0–28.9]	2.0 [0.0–11.8]	0.760

Table showing the mean and 95%-CI of the training and test dataset for females. Neither the age nor any of the calcification volumes were significantly different between the training test and test dataset (all $p > 0.05$ Mann-Whitney U-test)

The lSVR was able to outperform the baseline model on both the training dataset ($p < 0.001$) and the test dataset ($p = 0.002$). The MAE on the training dataset was 4.17 years and on the test dataset 4.00 years. Although the performance was better on the test dataset, this was not significant ($p = 0.123$).

Females had a vascular age that was, on average, 1.3 years lower than their chronological age [95%-CI 14.7 younger to 7.1 years older]. A histogram of the difference between chronological age and vascular age is shown in Figure 7.3.

Figure 7.3: Histogram of age differences in females



Histogram showing the difference in chronological age and vascular age in years for females. A negative value indicates decreased aging.

7.3.2 Males

A Mann-Whitney U-test showed no statistically significant difference between the training and test datasets. (see Table 7.6)

The lSVR was able to outperform the baseline model on the training and test datasets (both $p < 0.001$). The MAE on the training dataset was 4.11 years and on the test dataset 3.95 years. Just as for females, this difference in performance on the two datasets was not significant ($p = 0.417$).

On average, males were estimated to be 1.3 years younger than their chronological age [95%-CI 13.1 years younger to 6.7 years older]. A histogram showing the difference between vascular age and chronological is shown in Figure 7.4.

7.3.3 Sex Differences

Differences in the Sex Dedicated Models

The lSVR's feature weights indicate that sex is related to the importance of the vascular sites for determining vascular age. (See Figure 7.5). For females, compared to males, AAC, ECAC, and VBAC-volumes were more important. CAC and ICAC-volumes were

Table 7.6: Comparison of the training and test dataset

Characteristic (Males)	Training dataset (n=638) Mean [95%-CI]	Test dataset (n=202) Mean [95%-CI]	p-value
Age	68.3 [60.8–81.9]	68.0 [61.0–80.3]	0.429
CAC	364.5 [0.0–1918.7]	343.7 [0.0–1558.0]	0.644
AAC	653.9 [0.0–4202.9]	655.9 [0.0–4606.1]	0.791
ECAC	113.4 [0.0–721.0]	108.4 [0.0–598.6]	0.399
ICAC	120.4 [0.0–618.0]	108.8 [0.0–649.5]	0.539
VBAC	5.3 [0.0–50.7]	3.1 [0.0–31.0]	0.417

Table showing the mean and 95%-CI of the training and test dataset for males. Neither the age nor any of the calcification volumes were significantly different between the training and test datasets (all $p > 0.05$ Mann-Whitney U-test).

more important for males than for females in determining vascular age. The greatest relative sex differences were found in the ECAC (3.6 times more important in females) and the VBAC (2.6 times more important in females). The most important site for determining vascular age for either sex was the aorta arch.

There were no significant differences in the vascular age estimations of the sex-dedicated models. The distribution generated by the two models did not differ significantly either ($p = 0.995$). (See Table 7.7).

Table 7.7: Sex-dedicated model outcomes

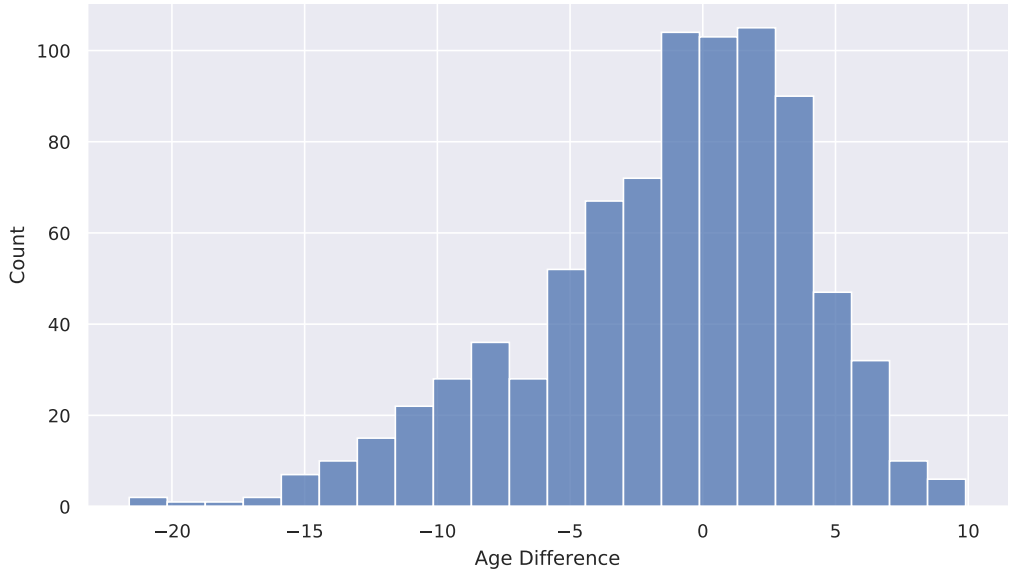
Characteristics	Female-specific Model Mean [95%-CI]	Male-specific model Mean [95%-CI]	p-values
Age Estimates (*years*)	66.8 [62.2–72.8]	66.9 [62.5–71.7]	0.178
Age Difference (*years*)	-1.3 [-14.7–7.1]	-1.3 [-13.1–6.7]	0.826
MAE (*years*)	4.13	4.07	0.996

The female-dedicated model and male-dedicated model showed no difference in vascular age. Age difference, as calculated by subtracting vascular age from chronological age, also showed no significant difference between the sex-specific models. The results shown here are a weighted average of the result on both the training and test datasets. The p-values were calculated using a Mann-Whitney U-test. It is important to note that this is not a direct comparison between females and males. Recall that these results were derived from fitting two different models to two different datasets. However, because there are no statically differences in the vascular age estimation of the models, we can compare the internals of those models better.

Differences in Sex in the Shared Models

The shared model, which has also been described before in Section 7.1, can be used to compare females and males directly. This shared model showed that females have a 0.69

Figure 7.4: Histogram of age differences in males



Histogram showing the difference in chronological age and vascular age in years for males. A negative value indicates decreased aging.

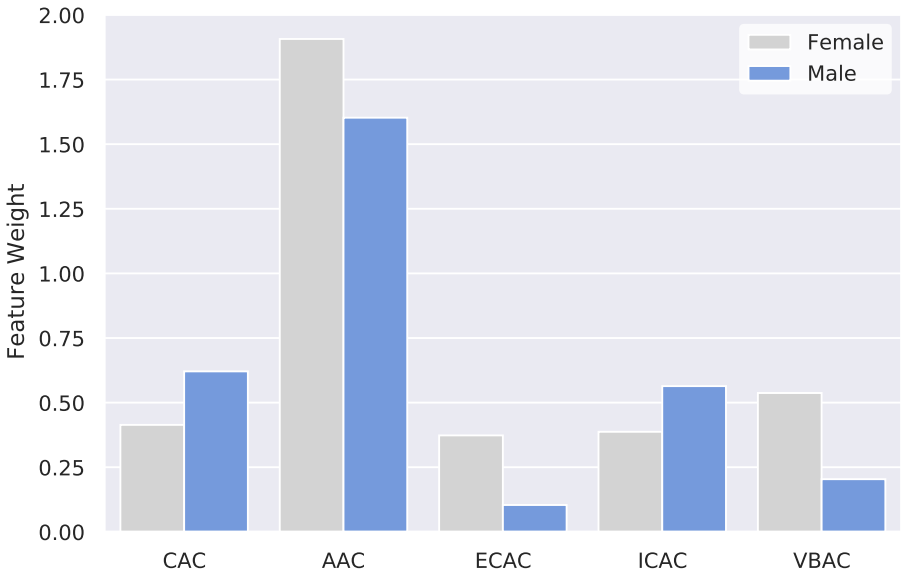
years lower vascular age than males. A distribution of vascular age, stratified per sex, is shown in Figure 7.6. The difference between vascular age and chronological age was also 0.61 years lower for females. (i.e. females showed healthier aging). The direct comparison between females and males is shown in Table 7.8.

Table 7.8: Direct comparison between females and males

Characteristic	Females mean [95%-CI]	Males mean [95%-CI]	p-value
Vascular Age	66.9 [62.5–72.2]	67.6 [62.7–73.0]	> 0.001
Age Difference	-1.3 [-14.4–7.1]	-0.7 [-11.8–7.4]	0.01

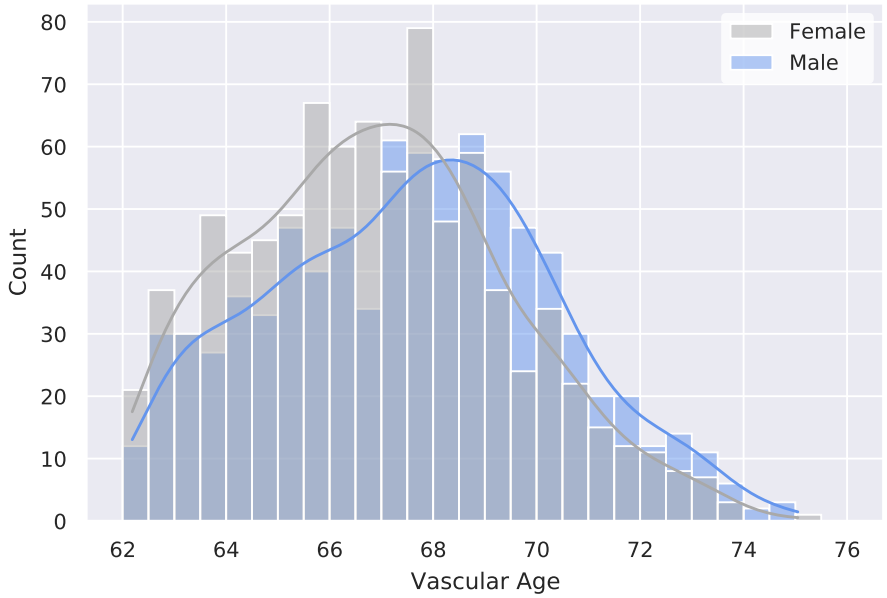
Females had a significantly lower vascular age and lower age difference.

Figure 7.5: Bar plot of feature importance for females and males



On the x-axis, the lSVR's inputs (i.e. features) are listed. The weights for each feature are attained by fitting an lSVR on a training dataset with females (gray) and one on a training dataset with males (blue). A higher weight indicates greater importance of that site in the determination of vascular age.

Figure 7.6: Distribution Plot of Vascular Age by Sex



Females (in gray) had a significantly lower vascular age than males (in blue).

Part IV

Epilogue

Chapter 8

Discussion

We discuss the results and limitations of the performed analyses and experiments in this chapter.

8.1 Discussion of the Findings

Research Question 1a
How much do calcification volumes at the different sites correlate with one another?

We found a moderate correlation between CAC, AAC, ICAC, and ECAC. VBAC showed a lower correlation with CAC, AAC, and ECAC. No site showed a negative correlation. These low to moderate positive correlation between the sites validate the idea that there might be local site-specific and vascular-system wide factors that cause these varying levels of calcification.

Research Question 1b
What is the correlation between age and arterial calcification volumes?

Individuals at higher ages had higher volumes of arterial calcifications. However, at any age, we found a great amount of individual variation at any site. The correlation between age and CAC, AAC, ICAC, and ECAC was moderate. The correlation between age and VBAC was the lowest of all the sites which might be explained by the high number of individuals with no VBAC even at higher ages. Calcification plots showed that, on average, calcification volumes increase linearly with age.

Research Question 1c
What sex differences exist in the calcification status of the different sites?

Females had significantly lower volumes of CAC, ECAC, ICAC, and VBAC. The greatest relative sex difference was found in the coronary arteries where males, on average, had over three times more CAC than females. Males also had over twice higher calcification volumes in the vertebrobasilar artery, intracranial and extracranial carotid arteries. One possible cause is that females have higher levels of the female sex steroid Estradiol. High endogenous Estradiol levels in postmenopausal females have been associated with significantly lower coronary calcification scores [58]. This might explain the difference in CAC because females have higher Estradiol levels.

Research Question 2a and 2b
(a) Do multi-site calcification models of vascular age contribute to a more accurate vascular age estimate compared to single-site (in particular CAC-only) models?
(b) What is the performance of single site models for the coronary arteries, the aortic arch, the vertebrobasilar artery, the extracranial carotid, and the intracranial carotid arteries, in the estimation of vascular age?

Previously, vascular age models only included the calcification of the coronary arteries [23, 59–64]. We found that models, to be precise, lSVRs, that used calcification volumes from multiple sites can more accurately predict an individual's age than single-site models can. The multi-site model had an MAE of 4.12 and 4.00 years on the training and test dataset respectively. However, the CAC-only model performed significantly worse with an MAE of 4.47 and 4.25. The ICAC-only model also performed significantly worse than the multi-site model with an MAE of 4.40 years on the training dataset and 4.22 years on the test dataset. The same held for the VBAC-model which had an MAE of 4.54 years on the training dataset and 4.38 years on the test dataset.

Only the AAC-only model came close to the multi-site model because it did not underperform compared to the multi-site model on the test dataset with an MAE of 4.03. However, this advantage of the AAC-only model over the other single-site models was lost on the training dataset because, with an MAE of 4.19 years, it performed significantly worse than the multi-site model. We did not compare the ECAC-only model with the multi-site model because it did not perform significantly better than the median estimator (the baseline model).

Research Question 2c
What is the impact of sex on the estimation of vascular age?

We found that AAC was the most important feature both for females and males in the models trained exclusively on males and females. This is in line with our earlier findings because the AAC-only model came closest to the performance of the multi-site models. This was not completely unexpected because AAC showed the strongest correlation with age (Spearman correlation coefficient $\rho = 0.43$).

However, the relation between a higher age-calcification-correlation and greater feature weight did not hold universally. ECAC had a higher correlation with age than CAC and VBAC, however, ECAC was the least important feature for both females and males in determining vascular age. When comparing the sex-specific models directly, we found that AAC, ECAC, and VBAC were relatively more important for females, and CAC and ICAC were more important for males.

Models trained on both females and males showed that each sex had a slightly lower vascular age than chronological age. This difference was more pronounced in females who had, on average, a 1.3 year lower vascular age compared to 0.7 years lower for males.

8.2 Threats to Validity

Although we studied a large cohort, it was unlikely to be a representative sample of the general population. The participants we studied had no contraindications to receive both a brain-MRI and two CT-scans. This might have introduced a selection bias towards healthy and able participants, this is also known as the healthy cohort effect [65]. Note that we only included participants that received an MRI because we had the ambition to study brain-age as well. Unfortunately, due to time constraints, we did not manage to do so. As a result, these results might not generalize to individuals who are unable or unwilling to undergo MRI and CT-scans.

We studied the participants in a retrospective cross-sectional manner. Individuals with higher CAC-levels have an increased risk of mortality and morbidity [66]. It is plausible that older individuals with higher calcification levels who were initially eligible to participate died or became too ill to participate. This type of selection bias is known as survivorship bias. Survivorship bias is likely to have downplayed the importance of calcifications in the sites that directly impact an individual's health and vise versa. For example, we found that ECAC had the lowest importance in determining the vascular age of females and males. However, high ECAC has been found to be an important risk factor for all-cause mortality with a hazard ratio of 1.31 in females and 1.43 in males [67].

In other publications [59–61], the vascular age of an individual was calculated from CAC-levels. This is known as a value-based approach [8] because calcification values are directly used to compute an individual's vascular age. Typically the chronological age of reference individuals is used to determine if someone has above or below average calcification levels for their age. We extended this approach by training models that use calcification volume values of multiple sites. A limitation of this approach is that chronological age is not a perfect metric to measure life-expectancy or quality of health [20]. Future studies can address this by including health outcomes in their follow-up, such as mortality and cardiovascular incidents, which can then be used to derive vascular age. More can be read about future studies in Section 10.1.

We used lSVRs to compute vascular age. The lSVRs performed better on the test data than on the training data which indicates underfitting. Although this difference was not statistically significant, we did see a tendency for the lSVRs to estimate that older individuals had a relatively younger vascular age. This might be partially explained by the survivorship bias and partly by the fact that linear models are not suited to find non-linear relations. Other, more complex models, might give a better fit for very old individuals.

The usage of calcification volumes to derive vascular age is both a strength and a weakness. On one hand, atherosclerosis is a life-long process in which atherosclerotic calcification is just one aspect. As a result, no precalcific atherosclerosis is taken into account in the estimation of vascular age. This makes calcification volume less suitable to estimate vascular age for younger individuals. In addition, the presence of calcifications tells us little about the etiology of those calcifications. On the other hand, calcifications are very strong predictors of health outcomes [64, 68] and they are more clinically relevant than other measures of atherosclerosis such as Carotid Intima Media Thickness (CIMT) [8].

Chapter 9

Related work

9.1 Biomarkers of Vascular Age

As described in Chapter 1, cardiovascular risk has traditionally been calculated using the Framingham risk factors. Vascular age, based only on the Framingham risk factors, can be established by comparing the risk of an individual to reference age groups. The vascular age is determined by finding the age group that has the same average risk as that individual.

In this section, we describe the related work that looked into biomarkers that aim to improve vascular age estimation.

9.1.1 CIMT

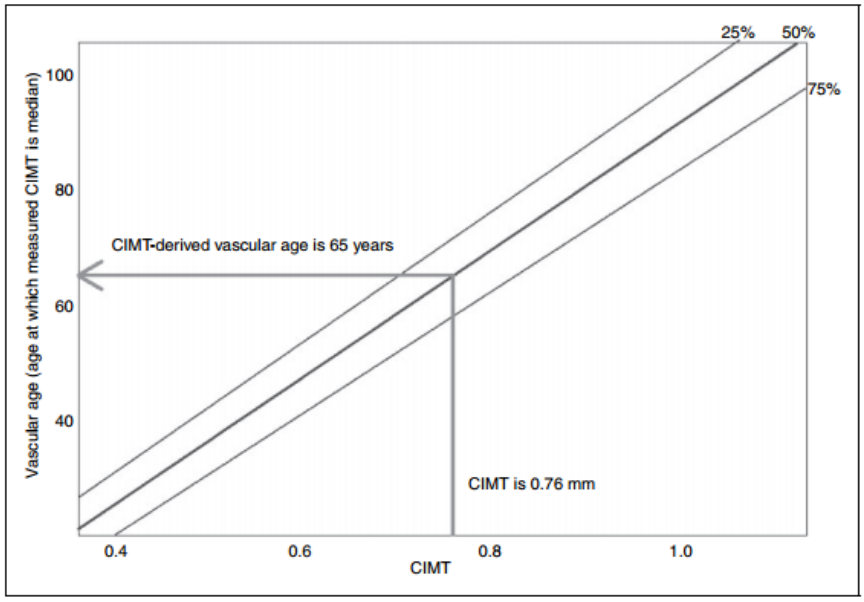
A previously often studied imaging approach to estimate vascular age included measuring the Carotid Intima Media Thickness (CIMT) [8].

The CIMT value of a patient is commonly compared to the median CIMT value of different age groups to derive a vascular age. (Figure 9.1)

Den Ruiter et. al. found that CIMT only provides a small, and likely clinically irrelevant, improvement when included in a Framingham Risk Score [69]. Costanzo et. al. questioned the usefulness of CIMT. He found that a treatment-induced regression in CIMT does not reduce the risk of cardiovascular events [70]. A 2012 meta-analysis published in the JAMA, concluded that CIMT measurements should not routinely be performed in the general population because the added value is too limited to lead to health benefits [71].

Thus other, more promising, biomarkers of vascular age are needed.

Figure 9.1: An example of deriving vascular age from a CIMT value.



Source: [8]

9.1.2 Coronary Artery Calcification

As mentioned before, CAC-derived vascular age has been used to improve cardiovascular risk estimation. When such a vascular age is added to a traditional risk estimation score, the ROC-AUC for coronary-related cardiovascular incidents increases from 0.76 to 0.83 [64]. This improvement has been shown to lead to better risk estimation and different prevention strategies for individuals [8, 72]. Although a great improvement, given the global impact of CVD is, there is a lot to be gained from more accurate risk prediction.

Additionally, CAC has only been shown to improve the risk estimation for coronary-related cardiovascular incidents [8]. CAC is not significantly correlated with stroke occurrence after correcting for carotid calcification volumes [10]. Thus for effective stroke prevention, which makes up 35% of all cardiovascular deaths, using calcification information from other sites ought to be considered [1].

9.1.3 Pulse Wave Velocity

In this subsection, we first describe what Pulse Wave Velocity (PWV) is. We finish by describing the related work that tried to used PWV to improve the models of vascular

age.

PWV is the velocity of a blood pressure pulse, generated by a heartbeat, moves through the blood vessels. PWV is calculated as the distance traveled by the pulse wave divided by the time taken to travel that distance:

$$\text{PWV} = \frac{\Delta d}{\Delta t} \tag{9.1}$$

PWV is used clinically as a measure for arterial stiffness where a higher PWV is indicative of a less distensible (i.e more rigid) vessel. To measure arterial stiffness, carotid-to-femoral pulse wave velocity (cfPWV) is considered to be the gold standard. One approach to cfPWV is to take the following steps:

- Two locations are picked where we can measure the carotid-pulse and the femoral-pulse.
- The distance between the two locations is measured.
- An ECG-monitor is applied.
- Tonometers are placed at the two locations to register the pulse wave.
- A PWV device and software (e.g. from SphygmoCor) can automatically calculate the PWV.

Cecel et. al. found that PWV increases with higher age (r=0.90) and blood pressure (r=0.91) [73, 74].

Kim et. al. found that PWV correlates with coronary artery disease (r=0.838, p=0.001) [75]. PWV is also associated with calcifications in both the small and large brain vessels and with cognitive decline in the elderly [75]. However, the underlying mechanisms that link arterial stiffness and atherosclerosis are not well-elucidated [75]. Additionally, PWV is a predictor of an increased Carotid Intima Media Thickness (CIMT), which has been discussed before.

Advantages of PWV are:

- It is highly predictive of cardiovascular events, independent of the Framingham risk factors (excluding blood pressure and age) [75]. For reference, the cfPWV in healthy individuals is $7.7 + 1.1 m/s$ [76] and a cfPWV of $> 10 m/s$ is a strong risk factor for cardiovascular disease [75].[1] A cfPWV increase of 1 m/s correlates with a 12% increased risk of cardiovascular disease [76].
- It can be performed non-invasively [73].

[1] Note that velocity normalization might be needed when comparing different studies [74]

- It is highly reproducible [74].

Multiple methods of estimating vascular age from PWV have been proposed in the literature. Groenewegen et. al. classified these methods into 2 groups [8]:

1. Risk-based methods
2. Value-based methods

In a risk-based approach, PWV is seen as a risk factor for cardiovascular events. Here, one derives a vascular age by finding the age group that has, on average, the same risk as a patient. This approach can be used with PWV as the only risk factor or by combining other risk factors, such as those from the Framingham risk score.

In a value-based approach, we use the velocity found from the PWV-measurement. We compare the patient's PWV to the average PWV per age group to find the vascular age.

PWV is the only method described in the systematic review on vascular age by Groenewegen et. al. that takes multiple arteries into account for deriving vascular age [8]. However, PWV is limited by the fact that it only looks at the vascular stiffness over a long vessel trajectory. Therefore, in contrast with calcification volumes at spatially distinct sites, PWV does not give localized information about the vessel quality.

Chapter 10

Conclusion

In this research, we calculated the vascular age of individuals in a large cohort by estimating their chronological age based on arterial calcification volumes. We found that incorporating the volumes of arterial calcifications of multiple sites leads to a better estimation of age compared to single-site analysis. These findings could have important implications for clinical practice in the future because better age estimations could be used to improve and personalize care. Nevertheless, more research has to be performed before we reach that point.

Data analyses showed great differences between and within individuals. Although calcifications increased linearly with age, younger individuals may have high levels of calcification and older individuals may have low levels of calcification. Additionally, the correlation between the calcifications at different sites is only low to moderate. Females showed lower calcification volumes at all sites compared to males however this difference varied per site.

To the best of our knowledge, this is the first study that incorporates calcification volumes from multiple sites in the estimation of an individual's age. Previously, only coronary artery calcification was accounted for. We ran linear support vector regressions for the final analyses because for this exploratory research we wanted the results to have a high level of explainability. We feel that more refined techniques could provide better estimates but at the cost of becoming less understandable to all but the most arduous statisticians and machine learning experts. We provide suggestions for these techniques in Subsection 10.1.3.

When comparing the models trained on only one sex, we found that calcification at a particular site does not contribute equally to vascular age for each sex. We found that both females and males had a lower vascular age than chronological age which indicates

healthy aging. However, this effect was more pronounced in females.

10.1 Future work

10.1.1 Increase Sites

We used information from five sites to build vascular age models. We found that the sites do not contribute equally to the estimation of vascular age. Other sites could be added to make the estimation more accurate. One such site could be the aortic heart valve because of its relation to aging [77]. Additionally, calcification of the aortic heart valve can cause stenosis, which is the leading cause of cardiac valve replacement [78].

Another approach could be to split up certain sites. For example, we used an aggregated value for the coronary arteries. Follow-up research could split up this site into separates sites for the right coronary artery, the left (common) coronary artery, the left anterior descending artery, and the left circumflex artery.

10.1.2 Risk-Based Approach

We build models that used chronological age as their label. However, as mentioned before, chronological age is not a perfect proxy for life-expectancy or health. Future studies could address this by using outcomes such as mortality and cardiovascular incidents. We feel that a vascular age derived from such health outcomes is likely to provide better insights into the importance of each site because it reduces survivorship bias. Given a large cohort, with a sufficiently long follow up, different types of health outcomes could even be traced back to the calcifications at different sites.

10.1.3 More Complex Types of Analysis

We used lSVRs for the analyses. This is a machine learning model that is suitable for finding linear relations. Using the lSVR we showed that multi-site analysis indeed leads to more accurate age estimations compared to single-site analysis. We feel, however, that more complex, non-linear machine learning models, such as neural networks, could increase the estimation-accuracy. Unfortunately, these models typically have the disadvantage of being harder to understand and explain.

Moreover, the features that we used may be combined to give new features. To give one example, the calcification of different sites might be multiplied with each other to create new features. Completely new features, such as the shape of the calcifications, may be added as well.

10.1.4 Relation of Different Types of Biological Ages

We have focused exclusively on vascular age however other types of biological ages exist. By analyzing the relations between these types of biological ages we can get new insights into the aging process. We hypothesize that these biological ages are related in interesting ways. For example, an increase in vascular age could lead to poorer brain vascularization, which could result in an increased brain age. If a high vascular age leads to an acceleration in brain age a few years later, then clinicians could use high vascular age as a potential early warning signal in an otherwise mentally well-functioning patient.

Chapter 11

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Appendix A

Appendix

A.1 Software Versions

A listing of the software that was used to perform our analyses is shown in Table A.1.

Table A.1: Versions of the main software used

Software	Version
Python	3.8.2
JupyterLab	2.2.9
Matplotlib	3.3.3
NumPy	1.19.4
Pandas	1.1.4
Scikit-learn	0.23.2
SciPy	1.5.4
Seaborn	0.11.0

A.2 Models Explored During Preparation

A listing of the models that we explored is shown in Table A.2.

Table A.2: Listing of models explored

ML/Statistical Models
SVR with an RBF-kernel
SVR with a polynomial kernel
SGDRegressor
SGDRegressor using an epsilon insensitive loss function
SGDRegressor using a squared epsilon insensitive loss function
LinearRegression
LinearSVR
ElasticNet
Lasso

Scikit-learn models that were explored during our preparation. Except for the configuration that is mentioned here, the default settings were used.