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Fuzzy Logic Scoring System for the Assessment of Cardiovascular Health Status based on Arterial Stiffness

DOCTORAL THESIS

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STATUTORY DECLARATION

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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ABSTRACT

Cardiovascular diseases are the leading cause of death globally. This work presents a novel scoring concept and a novel scoring technique based on Fuzzy Logic to assess the cardiovascular health status of a subject for preventive examinations. The scoring system uses scores (from 0 to 10) based on indicators or markers of the cardiovascular system and accounts for age and gender of a subject. In addition, the scoring system accounts for the impact of confounders. Furthermore, this work presents a setup procedure of the scoring system for surrogate variables of arterial stiffness (peripheral pulse pressure (pPP), central pulse pressure (cPP), aortic pulse wave velocity (PWV) and central augmentation index (cAI)) derived from data of the Anglo-Cardiff Collaborative Trial database (4634 subjects). Four different age groups were defined (40-49 years, 50-59 years, 60-69 years and 70-79 years). Reference values ('normal' and 'high') for pPP, cPP, PWV and cAI separated for each age group and gender were calculated. The effect of blood pressure on PWV, and the effect of heart rate and body height on cAI were also investigated. In addition, the effect of three different risk factors (smoking, obesity and diabetes mellitus II) on score results was analyzed. It could be shown that smoking has a fundamentally different effect on surrogate variables of arterial stiffness than obesity or diabetes mellitus II. Therefore, cAI is a good indicator for middle-aged (40-59 years) smokers but is of limited value for elderly smokers (60-79 years), obese subjects or subjects with diabetes mellitus II. In addition, pPP should not be used as indicator for young smokers. Results show further that cPP and PWV can be used as indicators for each investigated risk factor over the whole investigated age range (40-79 years). In conclusion, scores calculated by the scoring system based on surrogate variables of arterial stiffness can give information on structural changes of the arterial system measured as a pathophysiological increase of arterial stiffness due to the impact of risk factors or genetic predisposition. Hence, this approach provides a further method for the quantification of the cardiovascular health status in addition to the assessment of cardiovascular risk. Furthermore, the scoring concept and the scoring system based on easily measurable surrogate variables of arterial stiffness could be used for screening procedures and preventive examinations, or it could be implemented into devices.

Keywords: *peripheral pulse pressure, central pulse pressure, pulse wave velocity, augmentation index, early vascular aging, normal vascular aging, preventive examination.*

KURZFASSUNG

Kardiovaskuläre Erkrankungen stellen weltweit die häufigste Todesursache dar. Deswegen wurden auf Basis der Fuzzy Logik ein neuartiges Bewertungskonzept und eine neuartige Bewertungsmethodik zur Erfassung des kardiovaskulären Gesundheitszustandes entwickelt. Das darauf aufbauende Bewertungssystem berechnet eine Punktezahl (Score von 0 bis 10) auf Basis von nicht-invasiv erfassten Indikatoren des Herz-Kreislaufsystems und korrigiert die Berechnung für das Alter und Geschlecht der Versuchsperson. Zusätzlich korrigiert das Bewertungssystem den Einfluss von Störgrößen. Das entwickelte Bewertungssystem wurde anschließend für nicht-invasiv erfasste Messgrößen der arteriellen Gefäßsteifigkeit (peripherer Pulsdruck (pPP), zentraler Pulsdruck (zPP), aortale Pulswellengeschwindigkeit (PWG) und zentraler Augmentationsindex (zAI)) verwendet. Dies erfolgte auf Basis der Anglo-Cardiff-Collaborative-Trial-Datenbank (4634 Probanden). Es wurden vier Altersgruppen (40-49 Jahre, 50-59 Jahre, 60-69 Jahre und 70-79 Jahre) definiert und für jede Messgröße wurden Referenzwerte (‚normal' und ‚hoch'), gegliedert nach Altersgruppe und Geschlecht, berechnet. Zusätzlich wurde der Einfluss des Blutdrucks auf die Messgröße PWG sowie der Einfluss von Herzfrequenz und Körpergröße auf die Messgröße zAI untersucht. Einen weiteren Schwerpunkt stellte die Abschätzung des Einflusses von verschiedenen Risikofaktoren (Rauchen, Übergewicht und Diabetes Mellitus II) auf die Resultate des Bewertungssystems dar. Diese Untersuchung zeigte, dass sich Rauchen unterschiedlich auf die genannten Messgrößen der arteriellen Gefäßsteifigkeit im Vergleich zu Übergewicht und Diabetes Mellitus II auswirkt. Deswegen kann die Messgröße zAI als guter Indikator für Raucher mittleren Alters (40-59 Jahre) angesehen werden. Hingegen hat sich gezeigt, dass die Messgröße zAI ungeeignet für ältere Raucher (60-79 Jahre), übergewichtige Personen oder Personen mit Diabetes Mellitus II ist. Aufgrund der Ergebnisse kann auch darauf geschlossen werden, dass die Messgröße pPP kein guter Indikator für Raucher mittleren Alters (40-59 Jahre) ist und sich die Messgrößen zPP und PWG gut als Indikatoren für jede der untersuchten Risikogruppen eignen. Zusammengefasst: Mit dieser Arbeit konnte gezeigt werden, dass das Bewertungssystem auf Basis von Messgrößen der arteriellen Gefäßsteifigkeit dazu verwendet werden kann, Informationen über strukturelle Veränderungen des Gefäßsystems zu geben und dass sich strukturelle Veränderungen als pathophysiologischer Anstieg der arteriellen Gefäßsteifigkeit meist durch den Einfluss von Risikofaktoren oder genetischer Prädisposition manifestieren können. Deswegen könnte in Zukunft das vorgestellte Bewertungssystem, neben der etablierten Erfassung des kardiovaskulären Risikos, als zusätzliche Möglichkeit zur Erfassung des kardiovaskulären Gesundheitszustandes dienen. Folglich kann auch das vorgestellte Bewertungskonzept und die vorgestellte Bewertungsmethodik als neuartige und vielversprechende Methode angesehen werden, und das daraus entwickelte Bewertungssystem könnte für Screeningverfahren, Vorsorgeuntersuchungen oder in medizinischen Geräten Verwendung finden.

Schlüsselwörter: *Bewertungssystem, arterielle Gefäßsteifigkeit, Fuzzy Logik, peripherer Pulsdruck, zentraler Pulsdruck, Pulswellengeschwindigkeit, Augmentationsindex, frühzeitige Gefäßalterung.*

CONTENTS

LIST OF ACRONYMS

acronym	
ACCT	Anglo-Cardiff Collaborative Trial
AI	augmentation index
AUC	area under the curve
BMI	body mass index
cAI	central augmentation index
CHD	coronary heart disease
cPP	central pulse pressure
CVD	cardiovascular disease
DBP	diastolic blood pressure
DM II	diabetes mellitus II
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FIS	fuzzy inference system
FLSS	Fuzzy Logic Scoring System
HDL	high density lipoprotein
HR	heart rate
JNC7	The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
LDL	low density lipoprotein
MAP	mean arterial pressure
NCEP	National Cholesterol Education Program
PP	pulse pressure
pPP	peripheral pulse pressure
PWV	pulse wave velocity
RS-CC-System	reference value selection and confounder correction system
SBP	systolic blood pressure
TC	total cholesterol
TG	triglycerides
WHO	World Health Organization

LIST OF SYMBOLS

symbol	
physical variables	
A	area
E	elastic modulus
$\mathbf{E_{inc}}$	incremental modulus
F	force
P	pressure
Q	flow
t	time
V	volume
x	distance
ε	strain
σ	stress
variables used to describe the arterial system	
C	compliance
h	vessel wall thickness
l	vessel length
$\mathbf{l_0}$	initial vessel length
$\mathbf{p_d}$	diastolic blood pressure
$\mathbf{p_i}$	inflection point pressure
$\mathbf{p_s}$	systolic blood pressure
R	resistance
$\mathbf{r_1}$	inner vessel radius
$\mathbf{r_2}$	outer vessel radius
$\mathbf{Z_0}$	input impedance of the arterial tree
$\mathbf{Z_c}$	characteristic impedance
ρ	density of blood
Δl	change in vessel length

variables used for the Fuzzy Logic Scoring System	
b	reference value for confounder correction
r	reference value
μ	membership function
statistical variables	
P	percentile value
Q	quartile value
σ	standard deviation
Φ	area under the standard normal distribution
$\tilde{\mu}$	median
μ	mean

1. Introduction

1.1. Background

1.1.1. The role of cardiovascular diseases

Cardiovascular diseases (CVD) are the leading cause of death globally. The World Health Organization (WHO, 2011a) estimated that 17.1 million people died from CVD in 2004, representing 29% of all global deaths and of these deaths, an estimated 7.2 million were due to coronary heart disease (CHD) and 5.7 million were due to stroke. In the European Union CVD accounted for two million deaths and over 126 million hospital bed days, representing 277 hospital bed days per 1000 population (Leal et al., 2006). Furthermore, this statistic (data of 2003) shows that the CVD costs for the EU health care system was €105 billion and the total costs for the whole EU economy were €169 billion: approximately 62% were due to healthcare, 21% due to productivity losses, and 17% due to informal care (Leal et al., 2006).

1.1.2. Prevention of cardiovascular disease

CVD is a term for a variety of diseases of the heart and blood vessels. Most of the research in cardiovascular prevention is focused on the prevention of cardiovascular events such as CHD and stroke. The intensive research of the last decades provides an extensive amount of information on the impact of risk factor exposure such as smoking, obesity, diabetes mellitus or physical inactivity on the individual risk of a person for a cardiovascular event. In addition, a variety of non-invasive measurements are in focus of intensive research to provide additional information about the individual cardiovascular risk or health status of a person. Examples of non-invasive measurements are:

- *blood indicators* (e.g. total cholesterol (TC), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, blood glucose, HbA1c-value and homocysteine),
- *indicators derived from blood pressure measurement* (e.g. pulse wave analysis, ankle-arm-index, pulse pressure (PP), systolic (SBP) and diastolic blood pressure (DBP)),
- *indicators of structural changes of the arterial vessel wall* (e.g. flow mediated dilatation, intima media thickness and pulse wave velocity) and
- *structural changes of the heart* (e.g. left ventricular mass).

1.1.3. The role of risk scores

A variety of CVD risk, CHD risk and vessel age scores such as those based on the Framingham Study (NCEP, 2002; D'Agostino et al., 2008), the PROCAM score (Assmann et al., 2002) or the SCORE project (Conroy et al., 2003; Cuende et al., 2010) were developed to provide information about the level of risk faced by an individual. The benefit of using such scoring systems is, of course, that higher scores send an important signal to 'at-risk' individuals to modify their lifestyle in order to avoid cardiovascular events. For instance, the Framingham risk score provides information on the 10-year

risk of having a heart attack (NCEP, 2002). The Framingham risk score is calculated by information about age, gender, TC, HDL cholesterol, smoking, SBP and medication status. The methodology of this kind of risk scores is based on longitudinal data on cardiovascular outcome and statistical methods. In conclusion, most established risk scores give information on the individual risk for a cardiovascular event in a specific time period.

1.1.4. The role of normal and reference values

Indicators and markers can give additional information regarding the health status, the individual risk or the biological age of a subject. For the practical use of indicators or markers it is important to define the normal range for each indicator. For instance, hypertension is an important determinant of the development of CVD such as heart attack or stroke. Therefore, a variety of guidelines (e. g. WHO hypertension guidelines (Chalmers et al., 1999), JNC7 hypertension guidelines (Chobanian et al., 2003), ESH/ESC hypertension guidelines (Mancia et al., 2007)) define different classes for blood pressure. However, the goal of hypertension treatment is to lower blood pressure to decrease the individual risk for the development of CVD. In conclusion, it is important to define the normal range for each indicator and marker. Therefore, guidelines are the basis for diagnosis and treatment.

1.2. The aim of the thesis

The aim of this thesis was to develop a mathematical tool to estimate the cardiovascular health status of a person with non-invasive techniques or measurements. The model should estimate the extent of the progress of cardiovascular markers or indicators to prevent cardiovascular events in an early stage. The development of the mathematical tool was focused on arterial stiffness.

1.3. The concept of the thesis

A scoring concept was developed, and further implemented and programmed for markers and indicators of the cardiovascular health status and respective CVD. The resulting scoring system was designed to calculate easily interpretable scores ranging from 0 to 10. In addition, the impact of aging and confounders on the measurement of non-invasive indicators was taken into account for the score calculation. Furthermore, the score calculation was developed with the use of fuzzy logic and the scoring system was designed in an adaptable manner. Therefore, the score calculation can be used for different indicators or markers.

Furthermore, the developed scoring system was setup for surrogate variables of arterial stiffness (peripheral pulse pressure (pPP), central pulse pressure (cPP), pulse wave velocity (PWV) and central augmentation index (cAI)) and investigated for the impact of risk factors (smoking, obesity and diabetes mellitus II (DM II)) on score results. This was done based on data of 4634 subjects extracted from the Anglo-Cardiff Collaborative Trial (ACCT) database. Reference values for pPP, cPP, PWV and cAI were calculated and defined.

1.4. Arterial vessels

Arterial vessels are distensible conduits. The distensibility of the artery wall plays an important role in the interaction of the heart and systemic and pulmonary arterial systems in the circulation.

1.4.1. Arterial vessel wall structure

The arterial wall contains three distinct layers (see Figure 1). The inner layer is the tunica intima which consists of the vascular endothelium and the elastic lamina. The vascular endothelium is made of a single-layer of endothelial cells and a thin layer of elastin and collagen fibers. The outer layer is the tunica adventitia. This layer is a region of collagen and some elastin tissue. The tunica adventitia connects the arterial vessel with the surrounding tissue. The middle layer is the tunica media. This layer forms the large part of the wall and is the principle determinant of the mechanical properties of the vessel. The tunica adventitia has a fibrous structure and consists mainly of smooth muscle and elastin. (Nichols and O'Rourke, 1998, p77)

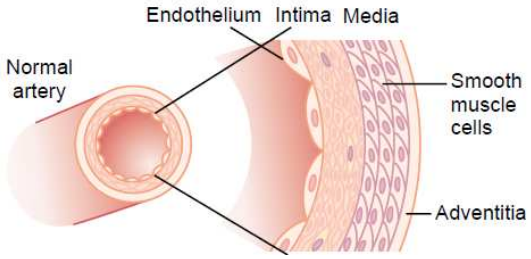


Figure 1: Structure of the arterial vessel wall. (partly reproduced from Guyton and Hall, 2006, p849; modified from Libby, 2002)

1.4.2. Mechanical properties of the arterial vessel wall

The mechanical properties of arterial vessels are predominantly defined by the amount of elastin, collagen and smooth muscle cells (Nichols and O'Rourke, 1998, p77). The amount of these components varies along the arterial tree. Central arterial vessels are more distensible than peripheral vessels. The amount of elastin decreases and the amount of smooth muscle cells increases with increasing distance from the aorta (Li, 2000). Consequently, the stiffness of arterial vessels increases along the arterial tree from the heart to the periphery. Therefore, central and large arteries can store a large amount of the stroke volume ejected by the heart.

1.4.2.1. Mathematical description of elasticity

A blood vessel segment can be described by the outer radius (r_2), the inner radius (r_1), the wall thickness (h) and the segment length (l) (see Figure 2).

$$E = \frac{\sigma}{\varepsilon} \qquad \textbf{(1)}$$

Elastic modulus: If stress is applied to a material it deforms. Therefore, the elasticity of a vessel segment can be mathematically described by the elastic modulus (E) which is defined as the resulting strain (ε) for a given stress (σ) (see (1)).

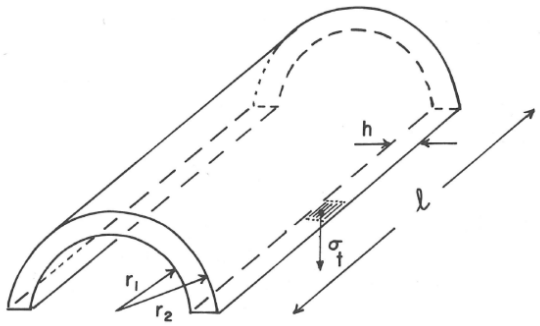


Figure 2: Model of the half of a segment of a blood vessel.
(reproduced from Noordergraaf, 1978)

Stress and strain: The stress results from an applied force (F) (see (2)). Hence, stress is defined as the force (F) per unit area (A) and has the same dimension as pressure (P). The deformation due to an applied stress is described as strain. That means strain defines the change in length (Δl) in relation to the initial length (l_0) of the body (see (3)). In conclusion, an increase in length is a positive strain and a decrease in length is a negative strain.

$$\sigma = \frac{F}{A} \qquad \textbf{(2)}$$

$$\varepsilon = \frac{\Delta l}{l_0} \qquad \textbf{(3)}$$

Young's modulus: The elastic modulus can be defined in different ways (Young's modulus, shear modulus, bulk modulus, longitudinal modulus), whereas the Young's modulus is the commonly used description of the elasticity of the arterial wall. However, the Young's modulus is a measure of the stiffness of the arterial wall and is defined as the ratio of tensile strain to tensile stress (see (4)).

$$E_x = \frac{\sigma_{xx}}{\varepsilon_{xx}} = \frac{Longitudinal\ force\ by\ unit\ area}{Extension\ per\ unit\ length} \qquad \textbf{(4)}$$

The Young' modulus can only be used if the material shows a linear-dependency between stress and strain as defined by the Hooke's law. The Hooke's law describes that a change in length (Δl) is linear depended to the force applied. This law holds for linear-elastic materials in a specific force range, but the stress-strain relationship of the arterial vessel wall is non-linear.

Non-linearity of the stress-strain relationship of the arterial vessel wall: For an artery segment the relationship between stress and strain is non-linear (Langewouters et al. 1984; Dobrin, 1986) and the elastic modulus increases with increasing stress (Patel et al., 1969). This result shows that Hooke's law

doesn't hold for arterial segments. However, Young's modulus is only applicable for a linear relationship between stress and strain. Therefore, an incremental elasticity modulus (E_{inc}) for non-linear relationships is used. The modulus is defined as the slope of the curve at a specific point (Nichols and O'Rourke, 1998, p57), calculated as a small change in stress ($\Delta\sigma$) divided by a small change in strain ($\Delta\varepsilon$) (see (5)).

$$E_{inc} = \frac{\Delta\sigma}{\Delta\varepsilon} \qquad \textbf{(5)}$$

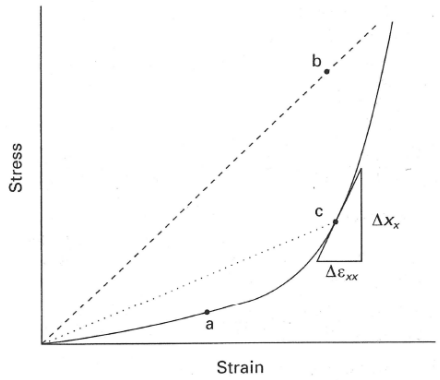


Figure 3: Relationship between stress and strain. Young's modulus can be applied at point a and b because of the linear relationship. For a non-linear relationship (point c) an incremental elasticity modulus is defined. (reproduced from Nichols and O'Rourke, 1998, p57; figure from Dobrin, 1978)

Anisotropy of the arterial vessels wall: Arterial vessels are distensible conduits and the arterial wall can be forced in longitudinal, radial or circumferential direction. However, the material properties are different for each direction due to the structure of the arterial vessel wall. That means arterial vessel walls are anisotropic (Li, 2000, p19). Patel et al. (1969) demonstrated for a dog aorta that the longitudinal elastic modulus is higher than the radial elastic modulus.

1.4.3. Windkessel model

The relationship of pressure (P) and flow (Q) in the arterial system can be described by a variety of models. The most commonly used model to describe the hemodynamic relationship in the arterial system is the Windkessel model as basically described by Frank (1899). Frank described a lumped parameter model which consists of two electrical elements (capacitor and resistance). In this model the total peripheral resistance (R) and systemic vascular compliance (C) is represented as a resistor parallel to a capacitor (see Figure 4).

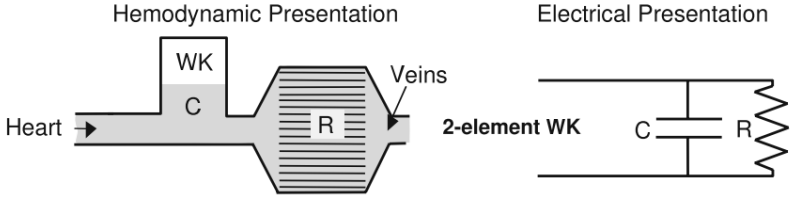


Figure 4: Two-element Windkessel model. The systemic compliance is modeled as capacitor and the total peripheral resistance as resistor. (adapted from Westerhof et al., 2009)

The compliance (C) is defined as the change in volume (dV) for a given change in pressure (dP):

$$C = \frac{dV}{dP} \quad \textbf{(6)}$$

The two-element Windkessel model of Frank was improved several times. For instance, a characteristic input impedance (Z_0) was added to the two-element Windkessel model. This approach connects the two-element Windkessel model with wave transmission models. (Westerhof et al., 2009)

1.5. Structural changes of the arterial vessel wall

As described (see Section 1.4.1), the inner lumen of the arterial vessel wall is coated with endothelial cells. The normal function of the endothelium is important because endothelial cells are involved in the regulation process of the vascular resistance through the release of nitric oxygen (Battegay et al., 2005, p10). That is, an increase in shear stress leads to the release of nitric oxygen which produces vasodilatation of a vessel segment. An impaired release of nitric oxygen is called 'endothelial dysfunction'. The endothelium plays an integral role in the regulation of vascular tone, platelet activity, leukocyte adhesion and thrombosis and is intimately involved in the development of atherosclerosis (Heitzer et al., 2001). Endothelial dysfunction was shown to be the first step to develop arteriosclerosis (medial changes) and atherosclerosis (intimal changes). Arteriosclerosis leads to a loss of distensibility and hardening of the arterial vessel wall and so influences blood pressure. This hardening process of the vessel wall leads to an increase of arterial stiffness. In contrast, atherosclerosis is the narrowing of the vessel lumen diameter and influences blood flow. In conclusion, arteriosclerosis and atherosclerosis leads to high blood pressure and impaired blood perfusion. Further these processes can lead to peripheral vascular disease and released plaques can lead to a vascular occlusion and respectively heart attack or stroke.

1.5.1. Arterial stiffness

The relationship between arterial stiffness and CVD has been the focus of intense research in the last two decades. Consequently, it is well accepted that an increase in arterial stiffness is associated with an increase in cardiovascular risk: Blacher et al. (1999a,b), Laurent et al. (2001), Boutouyrie et al. (2002), Mattace-Raso et al. (2006), Willum-Hansen et al. (2006), Mitchell et al. (2010), Vlachopoulos et al. (2010). Various surrogate variables including pPP, cPP, PWV and cAI can be measured easily and used to assess the effects of arterial stiffness (Laurent et al., 2006) and consequently to identify subjects at higher cardiovascular risk. It is also well established that arterial stiffness is a function of age (Mitchell et al., 2004; Benetos et al., 2002) and the progression of arterial stiffness can be negatively affected by a variety of lifestyle and disease factors such as smoking, obesity or diabetes mellitus: Benetos et al. (2002), Cruickshank et al. (2002), Mahmud and Feely (2003a, 2003b), Lacy et al. (2004), Vlachopoulos et al. (2004a, 2004b), Safar et al. (2006) and Jatoi et al. (2007). However, arterial stiffness is a result of structural changes in the vessel wall which is associated with an increase of collagen fibers and a decrease of elastic fibers (Nichols and O'Rourke, 1998, p73-97). This leads to

a lowering of vessel wall distensibility and a change of reflection phenomena in the arterial tree (Nichols and O'Rourke, 1998, p201-222).

1.5.2. Concept of normal and early vascular aging

As described, arterial stiffness increases progressively with age and this progression can be negatively affected by risk factors (see Section 1.5.1). Therefore it is important to distinguish between the physiological and the pathophysiological increase of arterial stiffness with age. For this reason further considerations propose to distinguish between normal vascular aging and early vascular aging: Nilsson et al. (2008, 2009) and Kotsis et al. (2011). For arterial stiffness, normal vascular aging can be defined by reference values of a 'healthy' group of subjects. In contrast, risk factor exposure leads to accelerated arterial stiffness progression and this can be defined as early vascular aging. Figure 5 presents the concept of early vascular aging adapted for arterial stiffness.

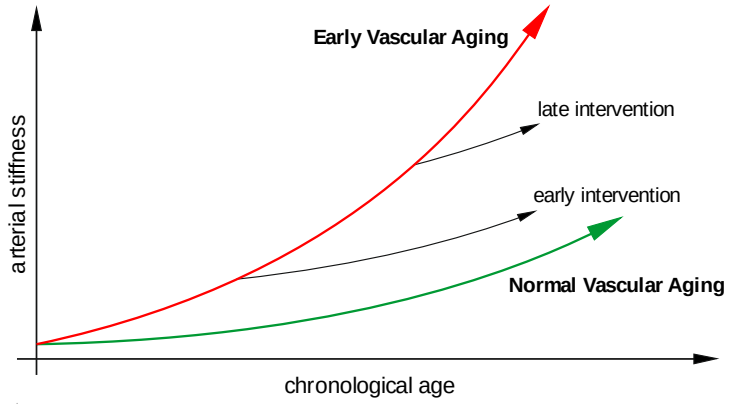


Figure 5. Concept of early vascular aging. (adapted from Nillson, 2009)

As shown in Figure 5 the concept of early vascular aging differentiates between normal progression of arterial stiffness (normal vascular aging) and abnormal progression of arterial stiffness with age (early vascular aging). This concept accounts for the impact of a lifestyle change due to an early or late intervention. A change in lifestyle (e.g. reduction of risk factors) leads to a lower progression of arterial stiffness with age. In addition, this concept can be further used to provide information on the biological age of a subject. Hence, if the calculated biological age is higher than the chronological age (age of the subject) the subject is prematurely aged and should reduce risk factors.

1.6. Introduction to Fuzzy Logic

Fuzzy Logic is an enhancement of the multi-valued logic. The basic of fuzzy logic was presented by Lofti Zadeh with the paper 'Fuzzy Sets' (Zadeh, 1965). The idea was to express vague conditions such as '*a little bit warm or a bit slow*'. The common binary logic is a two valued logic; only two conditions are possible: true or false, 0 or 1. In contrast to a binary logic, fuzzy logic can take every value between 0 and 1. That means every membership in a class is a matter of degree:

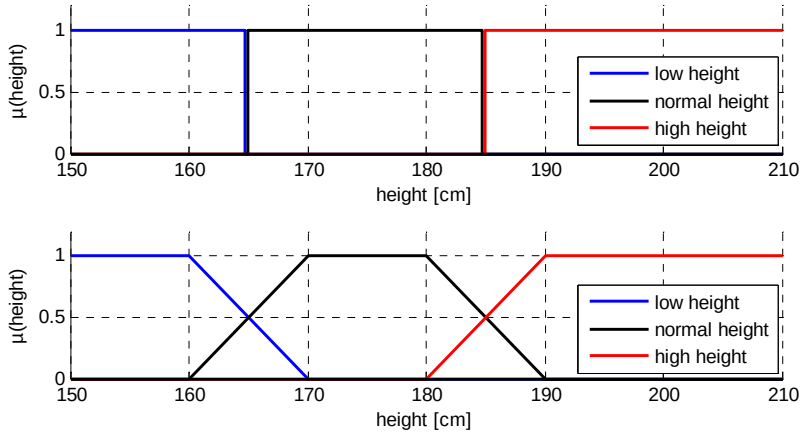


Figure 6: Binary logic (top graphic) vs. fuzzy logic (bottom graphic). In a binary logic (top graphic) every specific value of body height is related to one specific class ('low height', 'normal height' or 'high height') with a degree of membership of 1. In fuzzy set theory (bottom graphic) a specific body height value can be a member of more classes with a degree of membership between 0 and 1.

The degree of membership in a class is defined for every input value by a membership function (μ). The membership function can have diverse shapes such as triangular, trapezoid, gaussian or sigmoid. The example in Figure 6 (bottom) shows three membership functions which define the degree of membership for the classes (low height, normal height and high height).

The value range of a membership function is commonly defined by [0,1]. That is, every membership function μ of a fuzzy set maps elements of a given universal set X into real numbers [0,1] as shown in (7) (found in Klir and Yuan, 1995, p11).

$$\mu_A: X \rightarrow [0,1] \qquad (7)$$

The following equation (found and adapted from Klir and Yuan, 1995, p13) shows an example for the definition of a common triangular membership function. The variable h defines the point where the membership function reaches the maximum and the variable w defines the width of the triangular membership function. Figure 7 shows the plotted triangular membership function calculated with (8) for h=6 and w=1.

$$\mu(x) = \begin{cases} w(x-h)+1 & when \quad x \in \left[h-\frac{1}{w}, h\right] \\ w(h-x)+1 & when \quad x \in \left[h, h+\frac{1}{w}\right] \\ 0 & otherwise \end{cases} \qquad \textbf{(8)}$$

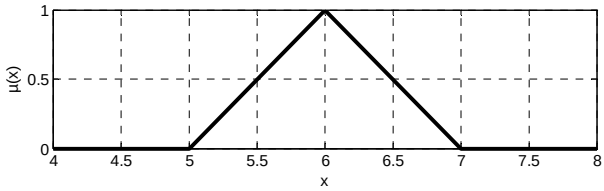


Figure 7: Triangular membership function.

Logical operators: Logical operations such as OR, AND, or NOT can be carried out on fuzzy sets. The logical operator AND (standard intersection) can be carried out by applying the MIN operator on the membership functions μ_A and μ_B (see (9), found and adapted from Klir and Yuan, 1995, p25). The logical operator OR (standard union) can be carried out by applying the MAX operator on the membership functions μ_A and μ_B (see (10), found and adapted from Klir and Yuan, 1995, p25). The logical operator NOT (standard complement) can be carried out by subtracting the membership function μ_A from 1 (see (11), found and adapted from Klir and Yuan, 1995, p25).

$$C = A \cap B \qquad \mu_c(x) = \min\ [\mu_A(x), \mu_B(x)] \qquad \textbf{(9)}$$

$$C = A \cup B \qquad \mu_c(x) = \max\ [\mu_A(x), \mu_B(x)] \qquad \textbf{(10)}$$

$$C = \bar{A} \qquad \mu_c(x) = 1 - \mu_A(x) \qquad \textbf{(11)}$$

The following figure shows the calculation principle of these three fundamental operators:

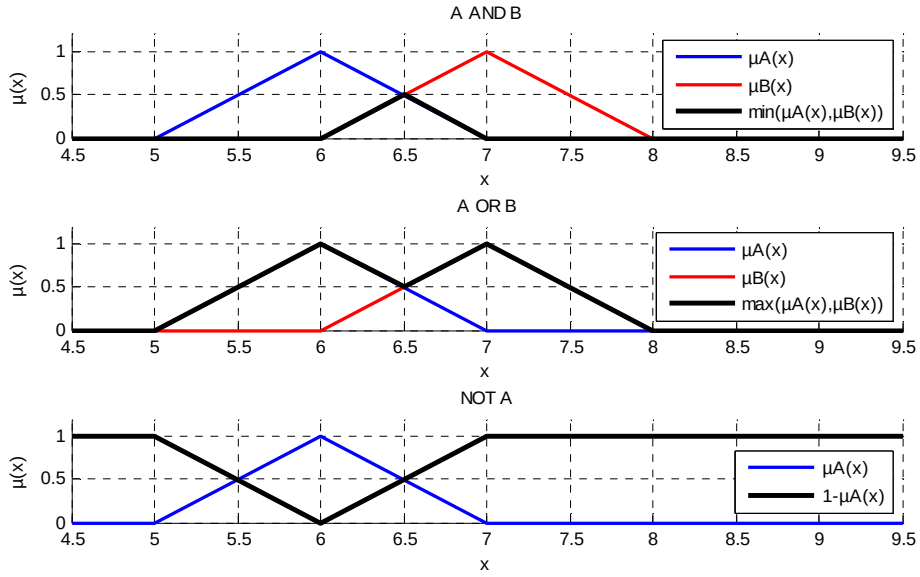


Figure 8: Logical operators in Fuzzy Logic. AND, OR, NOT.

1.6.1. Fuzzy Reasoning

Fuzzy reasoning is an important application of fuzzy logic and is based on expert rules to copy human knowledge and human decision making. Therefore, fuzzy reasoning is used for decision making, data classification, expert systems or pattern recognition. The fuzzy reasoning process uses inference rules expressed in IF-THEN format (Tanaka, 1997, p81). The IF-THEN rules are used to express linguistic statements between variables. An advantage of fuzzy reasoning is that relationships can be described in a 'linguistically way' (expert rules).

Simple example: Linguistic Statements (inference rules) for PWV and arteriosclerosis.

Rule1: IF pulse wave velocity IS high *THEN arteriosclerosis IS high* **(12)**
Rule2: IF pulse wave velocity IS normal *THEN arteriosclerosis IS normal* **(13)**
Rule3: IF pulse wave velocity IS low *THEN arteriosclerosis IS normal* **(14)**

The left part of the linguistic statement (IF) is called the antecedent block; the right part (THEN) is called the consequent block. Additionally, logical operators such as AND, OR and NOT can be used to generate linguistic statements.

1.6.1.1. Fuzzy inference system

A fuzzy reasoning process is mathematically realized by a fuzzy inference system (FIS). Several methods are available. In this thesis Mamdani's direct method (Mamdani, 1974; Mamdani and Assilian, 1975) was used. The following part shows the principle of a FIS with the Mamdani's direct method and is explained by an example adapted from Tanaka (1997, p86-p91). The example is based on two rules:

Rule 1: *IF x (input1) IS A_1* *AND* *y (input2) IS B_1* *THEN z (output) IS C_1* **(15)**
Rule 2: *IF x (input1) IS A_2* *AND* *y (input2) IS B_2* *THEN z (output) IS C_2* **(16)**

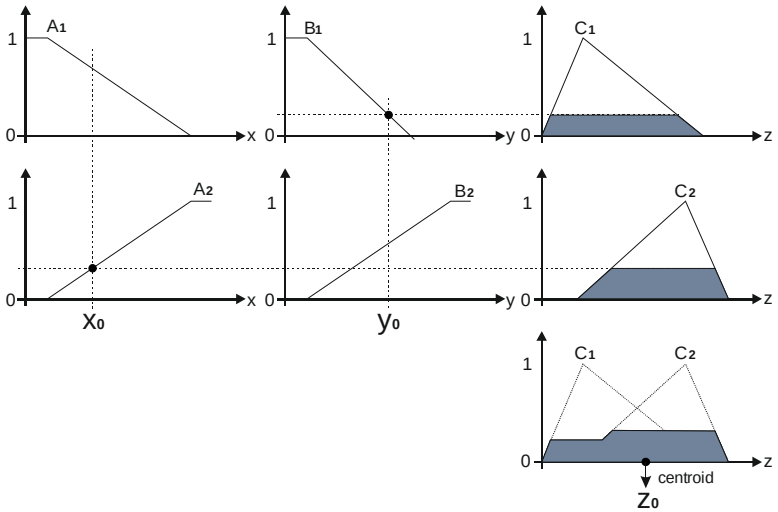


Figure 9: Example – Reasoning process with Mamdani's direct method.
(adapted from Tanaka, 1997, p87)

Figure 9 shows the reasoning process with Mamdani's direct method based on rule 1 (see (15)) and rule 2 (see (16)). The input variables are x and y; the output variable is z; the fuzzy sets are A_1, A_2, B_1, B_2, C_1 and C_2. Every fuzzy set is described by a membership function – for example: membership function μ_{A1} describes the fuzzy set A_1.

The Mamdani's direct method is based on five steps:

Step 1: fuzzification
Step 2: measurement of rule adaptability
Step 3: implication
Step 4: output aggregation
Step 5: defuzzification

Step 1: The first step of a fuzzy reasoning process is called fuzzification – for every input value (crisp numerical value) a degree of membership is determined (0 to 1). This relationship is described by a membership function (μ).

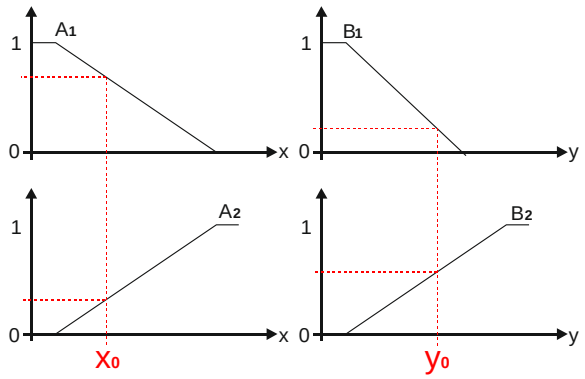


Figure 10: Step 1 – fuzzification. The degree of membership in A_1 and A_2 is determined for a specific input value x_0 and the degree of membership in B_1 and B_2 is determined for a specific input value y_0. The fuzzy sets A_1 and A_2 are described by the membership function μ_{A1} and μ_{A2} and the fuzzy sets B_1 and B_2 are described by the membership function μ_{B1} and μ_{B2}. (adapted from Tanaka, 1997, p87)

Step 2: is carried out to measure the adaptability (w) of each rule. The fuzzy sets in the antecedent part of rule 1 and rule 2 (see (15) and (16)) are connected by an AND operator. It follows as described in (17) and (18) (found and adapted from Tanaka, 1997, p86) that the MIN operator has to be applied on both determined membership values.

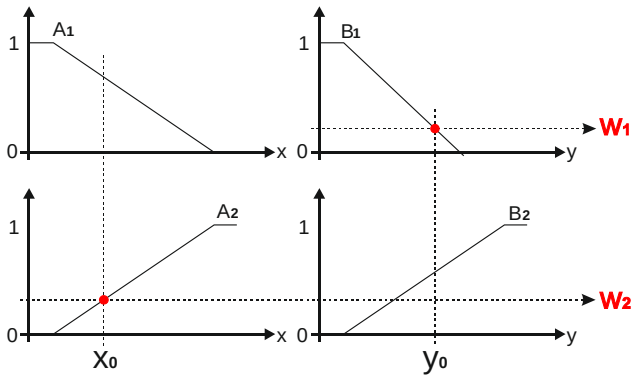


Figure 11: Step 2 – measurement of adaptability. The adaptability is measured by applying the MIN operator on the membership values of x_0 and y_0. (adapted from Tanaka, 1997, p87)

$$w_1 = \min\ [\mu_{A1}(x_0),\ \mu_{B1}(y_0)] \qquad \textbf{(17)}$$

$$w_2 = \min\ [\mu_{A2}(x_0),\ \mu_{B2}(y_0)] \qquad \textbf{(18)}$$

Step 3: The next step is called implication. Implication is the process of applying the adaptability obtained in step 2 to the fuzzy sets in the consequence part. This process is used to obtain the conclusion of each rule (Tanaka, 1997, p87). The input of the implication process is the antecedent output (adaptability) and the output of the implication process is a fuzzy set. Different implication methods are available. The two most important are the truncation method and the scaling method.

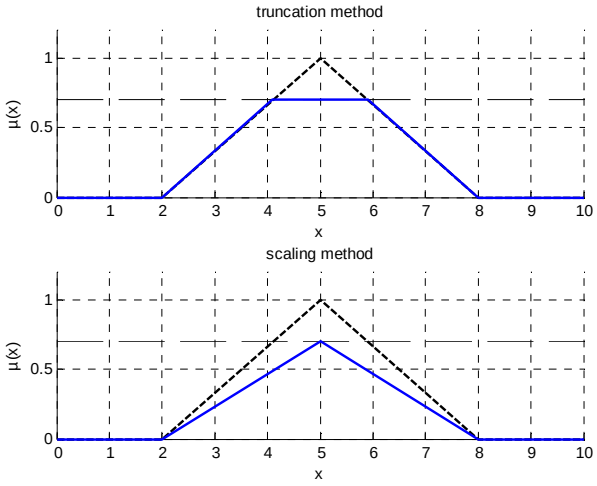


Figure 12: Truncation and scaling method. The truncation method truncates the consequent fuzzy set by the value calculated in the antecedent part. The scaling method scales the consequent fuzzy set to a maximum (value calculated in the antecedent part).

Figure 13 shows the result of implication (truncation method is used) for this example. The conclusion of rule 1 and rule 2 is calculated by (19) and (20) (found and adapted from Tanaka, 1997, p87):

$$\mu_{C\prime 1}(x_0) = w_1 \wedge\ \mu_{C1}(z) \qquad \forall z \in Z \qquad \textbf{(19)}$$

$$\mu_{C\prime 2}(x_0) = w_2 \wedge\ \mu_{C2}(z) \qquad \forall z \in Z \qquad \textbf{(20)}$$

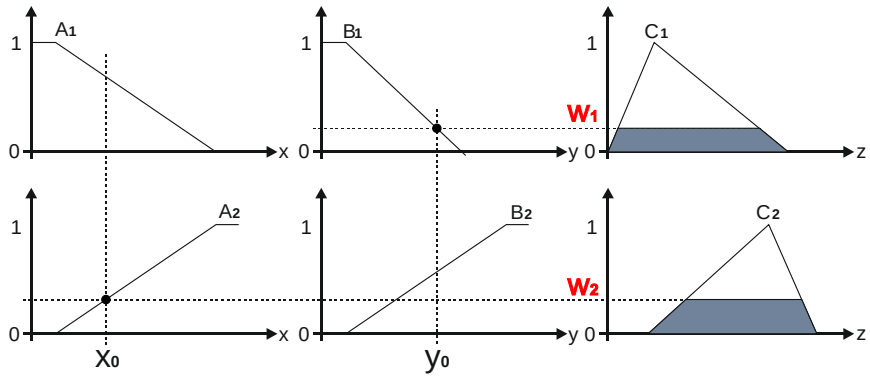


Figure 13: Step 3 – implication (truncation method) for rule 1. The fuzzy set C1 is truncated by the calculated adaptability value w_1 and the fuzzy set C2 is truncated by the calculated adaptability value w_2. (adapted from Tanaka, 1997, p87)

Step 4: is used to aggregate the conclusions of each rule. An aggregation of the truncated conclusion fuzzy sets is performed. Several methods can be used: maximum, probabilistic OR and summation (The MathWorks, 2010a, p2-25). The aggregation in this example was calculated by (21) (found and adapted from Tanaka, 1997, p87). Figure 14 shows the aggregated conclusion area.

$$\mu_C(z) = \mu_{C\prime 1}(z) \wedge \mu_{C\prime 2}(z) \qquad \forall z \in Z \qquad \textbf{(21)}$$

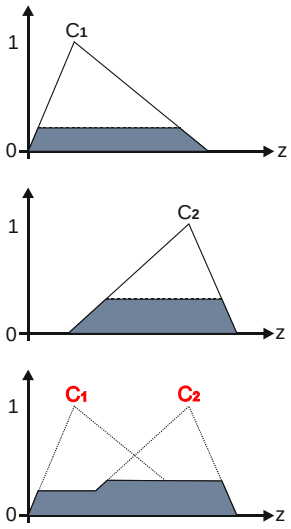


Figure 14: Step 4 – aggregation. The aggregation of the truncated conclusion fuzzy sets C_1 and C_2 is shown. (adapted from Tanaka, 1997, p87)

Step 5: The last step is called defuzzification. This step calculates a definite output value depending on the result of step 4 (aggregated conclusion fuzzy sets). Several defuzzification methods are known: centroid, bisector, middle of maximum, largest of maximum and smallest of maximum (The MathWorks, 2010a, p2-27). Figure 15 shows the defuzzification with the commonly used centroid method; following equation (found in Tanaka, 1997, p88) is used:

$$z_0 = \frac{\int \mu_C(z)\, z\, dz}{\int \mu_C(z) dz} \qquad \textbf{(22)}$$

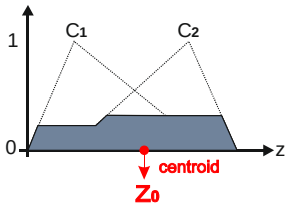


Figure 15: Step 5 – defuzzification. The defuzzification with the centroid method is shown. (adapted from Tanaka, 1997, p87)

1.7. Development process and structure of the presented work

Figure 16 shows the development process of the scoring system based on surrogate variables of arterial stiffness. As shown in Section 1.2, the aim of the thesis was to estimate the present cardiovascular health status of a subject with non-invasive techniques or measurements. Therefore a stepwise approach was used. The following sections give an overview of each step.

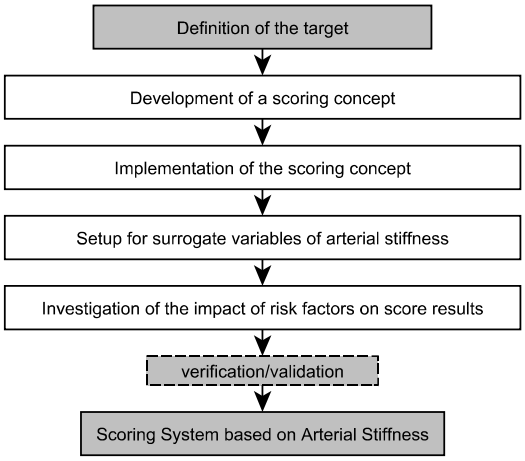


Figure 16: Development and structure of the presented work.

1.7.1. Development of a scoring concept

A scoring concept was developed based on the concept of normal and early vascular aging (see Section 1.5.2) to differentiate between a normal or abnormal value of an indicator or marker of the cardiovascular system. Basically, the scoring concept can be used for a variety of indicators or markers of the cardiovascular system. (see Chapter 2)

1.7.2. Implementation of the scoring concept

The scoring concept was implemented by using Fuzzy Logic. This technique was able to be applied due the fact that fuzzy logic can be used to describe the change of an indicator or marker as a smooth transition between a healthy state and a diseased state. The developed scoring system is called ‘Fuzzy Logic Scoring System’ (FLSS). (see Chapter 2)

1.7.3. Setup for surrogate variables of arterial stiffness

The scoring concept and the scoring system developed in this work can be used for a variety of indicators or markers of the cardiovascular system. The setup of the scoring system was focused on the arterial system and furthermore on arterial stiffness. However, arterial stiffness was used and investigated as input information due to the characteristics described below:

Surrogate variables of arterial stiffness derived from blood measurement such as pPP, from wave propagation characteristics of the arterial tree such as PWV, and from the central aortic pressure waveform such as cPP or cAI can be measured non-invasively. Hence, these surrogate variables are

easily assessable and suitable for preventive check-ups and screening examinations. It was also shown that arterial stiffness is an important biomarker that can reclassify risk. Vlachopoulos et al. (2010) presented a meta-analysis of 17 longitudinal studies and showed that aortic PWV, a measure of arterial stiffness, is a strong predictor of future cardiovascular events and all-cause mortality. However, the investigation of arterial stiffness and of surrogate variables of arterial stiffness is predominantly focused on the use for improving risk stratification. For instance, Wang (2011) discussed the possible enhanced role of assessment of PWV as a novel risk factor for cardiovascular events in addition to the assessment of traditional risk factors such as smoking, hypertension, DM II, dyslipidaemia, obesity or age. Wang (2011) also suggests that from the current knowledge of biomarkers that are able to reclassify cardiovascular risk, the only two potential candidates are coronary calcium score and aortic PWV.

However, it is important to note that the scoring technique presented in this work gives information related to the normality or abnormality of a variable at the time of measurement but it gives no information regarding cardiovascular risk. That is, this work presents an investigation of arterial stiffness for the use as indicator for pathophysiological changes of arterial elasticity due to the impact of traditional risk factors. Therefore, the working hypothesis described in the following section was defined and investigated.

1.7.3.1. Working hypothesis

It was hypothesized that surrogate variables of arterial stiffness show a different course with age for healthy subjects and for subjects exposed to risk factors. Furthermore, it was hypothesized that this information can be used to calculate scores which give information on the cardiovascular health status of the arterial system. In addition, it was hypothesized and investigated that the impact of traditional risk factors leads to a shift to higher score results for subjects at risk compared to healthy subjects.

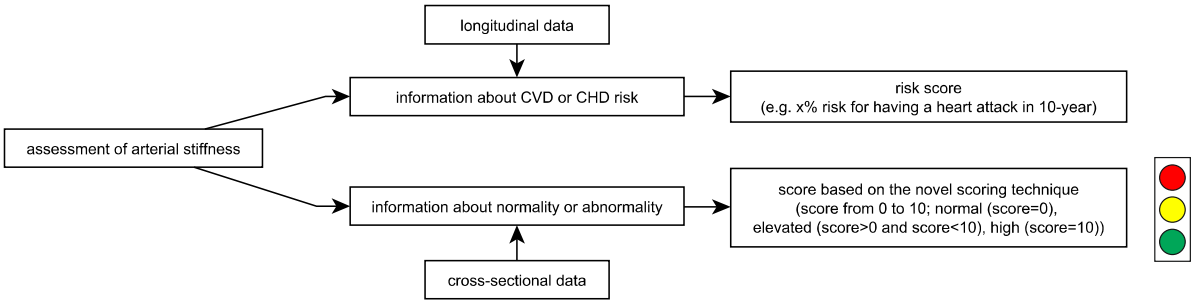


Figure 17: Differentiation between risk scores and scores calculated by the presented scoring system based on arterial stiffness. The calculation of risk scores is based on data of longitudinal cohort studies whereas the calculation of scores by the presented scoring technique is based on cross-sectional studies.

Hence, this work presents a scoring concept which is not focused on giving information specifically on cardiovascular risk. It is rather focused on giving information on the normality or abnormality of a variable at the time of measurement (see Figure 17). (see Chapter 3, 4 and 5)

1.7.4. Investigation of the impact of risk factors on score results

As described in the working hypothesis (Section 1.7.3.1), the impact of different risk factors (smoking, obesity and DM II) on surrogate variables of arterial stiffness and therefore on score results was investigated. Furthermore, an investigation was conducted on which surrogate variable of arterial stiffness respond more sensitively to a specific risk factor and if surrogate variables have a different performance as classification variables between middle-aged (40-59 years) and elderly subjects (60-79 years). (see Chapter 4)

1.7.5. Future work

A variety of further steps (e.g. additional validation and data) are necessary to establish the scoring system for screening procedures, preventive examinations or for the use in devices. (see Chapter 5)

2. Fuzzy Logic Scoring System

2.1. Introduction

A scoring system based on fuzzy logic (FLSS) was developed to estimate the cardiovascular health status of a subject based on indicators or markers of the cardiovascular system. Therefore, the scoring system was developed in an adaptable manner. That is, the scoring system can be used for different indicators or markers. Hence, the informative content of the calculated output score depends on the chosen input variables. This chapter shows the scoring concept and the implementation of the scoring concept based on Fuzzy Logic.

2.1.1. Required main features of the scoring system

Age-dependency of normal values: Most indicators or markers of the cardiovascular health status are age-dependent but this change can be physiological or pathophysiological. Hence, it is important to distinguish between a physiological and pathophysiological condition for each variable. Therefore, it is necessary to define separated normal values for different age categories.

Assessment of a pathophysiological change: The differentiation between normal and abnormal values is important for the early diagnosis of a pathophysiological change of a variable, but it is also important to assess how much a variable has changed compared to the normal level. For instance, the information that blood pressure has increased compared to normal values is not satisfactory. It is further important to give information about how much blood pressure has increased compared to normal values. However, the calculation of a score can give information on the relative change of a variable from normal values to abnormal values.

Confounders: Some measurements of indicators or markers are affected by confounders. Therefore a change of a variable from normal values to abnormal values can be due to impact of confounders. For instance, some measurements are blood pressure dependent. Therefore, a scoring system should account for the effect of confounders on measured indicators or markers.

Gender-dependency: Normal values may differ between male and female subjects. Therefore, a scoring system should use different reference values for male and female subjects.

2.2. Methods

2.2.1. Scoring concept

The scoring concept was designed based on the concept of normal and early vascular aging (see Section 1.5.2); and the required main features of the scoring system (see Section 2.1.1). Figure 18 presents the main principle of the scoring concept. For each input variable of the scoring system an area with ‘normal’, ‘elevated’ and ‘high’ values is defined by reference values. For most indicators or

markers of the cardiovascular health status these areas are shifted to higher values with age to account for the age-dependency. This shift is due to the physiological change of an indicator or marker with age. The borderlines between these three areas (‘normal’, ‘elevated’ and ‘high’) are defined by reference values. Most reference values are calculated for decades of age. Hence, a pair of reference values (‘normal’ and ‘high’) for each decade of age has to be defined. Additionally, separated reference values for male and female subjects have to be calculated to account for the impact of gender.

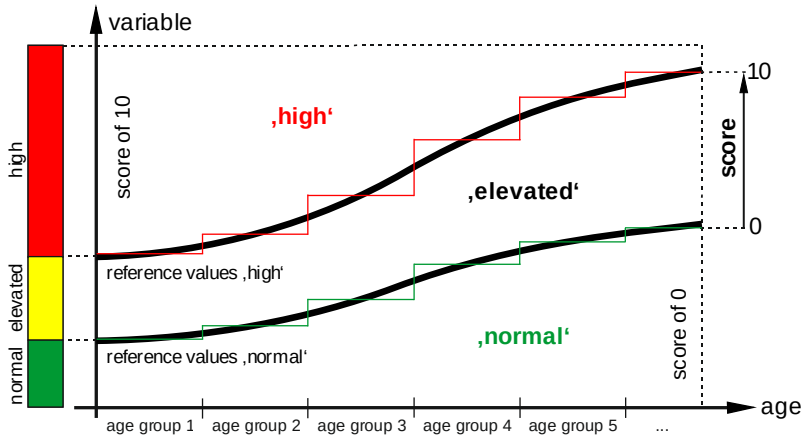


Figure 18: Scoring concept. Every input variable has a region of ‘normal’, ‘elevated’ and ‘high’ values. Borderlines are defined by age and gender-specific reference values. Scores are increasing from 0 to 10 between these two borderlines.

Score: The score ranges from 0 to 10 and gives information of the increase from ‘normal’ values to ‘high’ values. Therefore, the score increases from 0 to 10 between two reference values ‘normal’ and ‘high’. Hence, this concept can be compared with a traffic light system: A score of 0 equals a traffic light of ‘green’, a score greater than 0 and lower than 10 equals a traffic light of ‘yellow’ and a score of 10 equals a traffic light of ‘red’. In conclusion, the score value gives information about how much a variable has increased compared to normal levels.

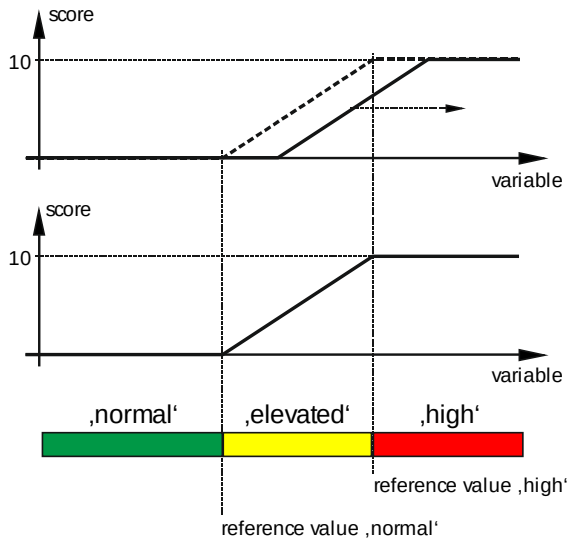


Figure 19: Score ramp for one age group. The score ramp (0 to 10) is defined by the reference values (‘normal’ and ‘high’) of a specific age group. The score increases from 0 to 10 between the two reference values (bottom diagram). Furthermore, the originated score ramp can be shifted to higher or lower values depending on the impact of confounders (top diagram).

Score ramp: For each decade of age a pair of reference values ('normal' and 'high') has to be defined. Between these two reference values the score increases from 0 to 10 and defines a score ramp as shown in Figure 19. For most indicators and markers the score ramp is shifted to higher values with increasing age. However, the shape of the score ramp can be defined based on the physiological behavior of an indicator or marker (e.g. linear, sigmoidal or other analytical functions).

Confounder-correction: Some measured indicators or markers show a confounder-dependency. To account for this situation it can be necessary to shift the reference values and respectively the score ramp to higher or lower values due to the impact of confounders. This is shown in Figure 19.

2.2.2. Implementation of the scoring concept

A scoring system (FLSS) was devolved and programmed based on the presented scoring concept. The mathematical implementation of the scoring concept was done based on the theory of fuzzy sets (Zadeh, 1965). The FLSS was programmed with MATLAB© software (R2008a, The MathWorks™).

2.2.2.1. Structure of the FLSS

Figure 20 shows the basic structure of the scoring system. The FLSS uses indicators or markers of a subject as input variables to calculate output scores. Additional information of the subject is used to correct the score calculation for age and gender; and further for the impact of confounders of the measurement. Each output score of a variable is calculated in relation to age- and gender-related pairs of reference values ('normal' and 'high'). Hence, a pool of reference values with separated reference values for each variable, decade of age and gender is used. The FLSS was developed based on a FIS, as described in detail in Section 1.6.1.1, and additional functionality was added on to derive the FLSS.

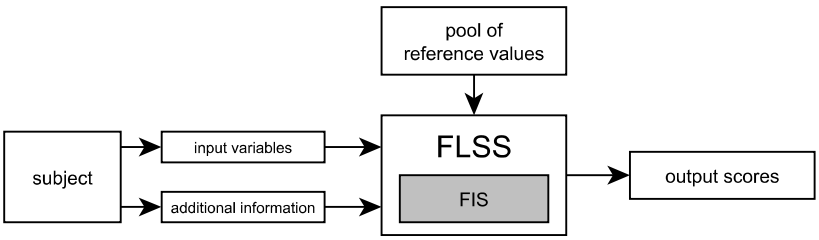


Figure 20: Basic structure of the FLSS. The FLSS is based on a FIS and calculates scores for markers or indicators of a subject in relation to reference values. The score calculation can be adjusted by additional information of the subject.

Figure 21 shows a more detailed structure of the FLSS. Input variables can be added on the scoring system and for each input variable a single output score ranging from 0 to 10 is calculated. Furthermore, weighted overall scores can be calculated based on a set of input variables. Therefore, adequate weighting factors have to be chosen. Scores are calculated by the *FIS*. The *reference value selection and confounder correction system* (RS-CC-system) is used to correct the score calculation for age and gender; and the impact of confounders.

RS-CC-System: Additional information (age, gender and confounders) is used to correct the score calculation. The RS-CC-System uses this information of the subject to extract the adequate age- and gender-related pair of reference values for each input variable from a pool of reference values. Regression equations are used to correct the impact of confounders by shifting the reference values to higher or lower values. Hence, this shift results in a shift of the membership functions in the FIS and a shift of the score ramp as shown in Figure 19. Therefore, adequate age- and gender-related regression equation for each indicator and confounder is extracted from the pool of regression equations. In conclusion, the FIS is used to calculate and weight the output scores and the RS-CC-System is used to correct the score calculation for age, gender and confounders.

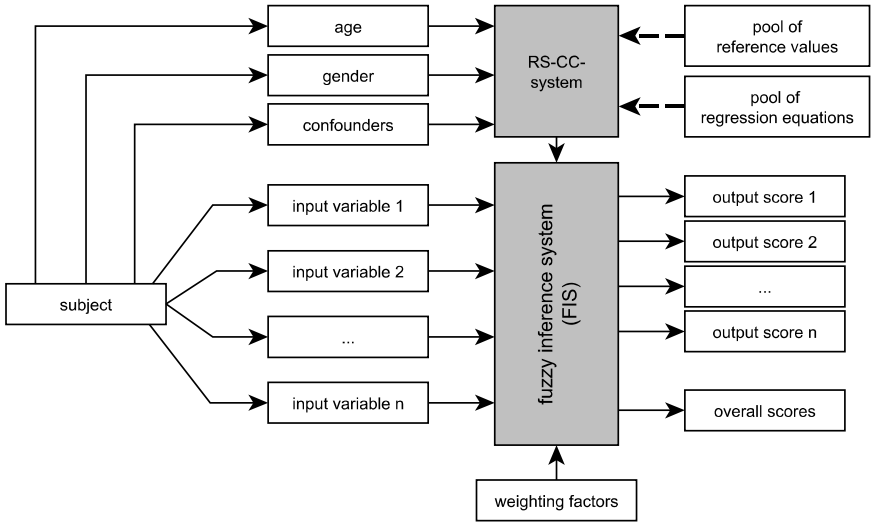


Figure 21: Structure of the FLSS. The FLSS consists of two sub-systems. First a FIS to calculate and weight the scores and second a RS-CC-system to correct the score calculation for age, gender and confounders.

2.2.2.2. Fuzzy inference system

The FIS is used to calculate scores for the input variables. The FIS was programmed with the Fuzzy Logic Toolbox™ (The MathWorks™) based on MATLAB© software (R2008a, The MathWorks™).

Mamdani's direct method is used and based on five calculation steps (fuzzification, rule adaptability, implication, aggregation and defuzzification). Different calculation methods are available for each step (see Section 1.6). The following list summarizes the calculation methods used:

AND method:	*'min'*
OR method:	*'max'*
Implication method:	*'truncation'*
Aggregation method:	*'sum'*
Defuzzification:	*'centroid'*

Figure 22 shows a flow chart of the calculation process based on Mamdani's direct method. The calculation process is defined by the chosen input and output membership functions; and further by the chosen linguistic rules and the weighting factors of the rules.

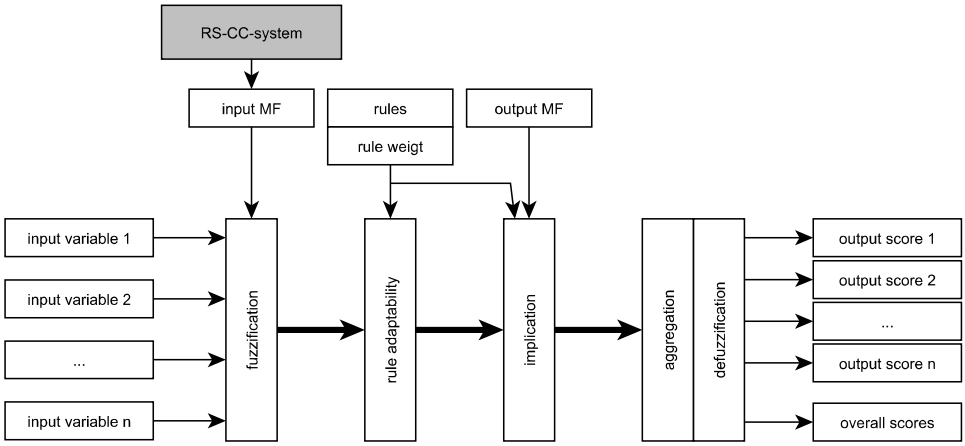


Figure 22: Structure of the FIS. Five calculation steps are necessary to calculate scores for the input variables. The fuzzification step can be used to correct the score calculation for age, gender and confounders. Weighted rules can be used to calculate weighted overall scores.

2.2.2.2.1. Input membership functions

The input membership functions are used for the fuzzification process of the FIS. They were normally defined as trapezoid functions. In combination with the triangular defined output membership functions, this leads to a nearly linear score increase from 0 to 10. However, the shape of the input membership functions can be defined in different ways, for example: linear or sigmoidal. In conclusion, the definition of the shape has a strong impact on the shape of the score ramp.

The increase of the score from 0 to 10 is defined by a pair of reference values ('normal' and 'high') as described in the scoring concept (see Section 2.2.1). Therefore, two input membership functions 'normal' and 'high' are defined based on the reference values. Figure 23 shows this procedure with trapezoid functions.

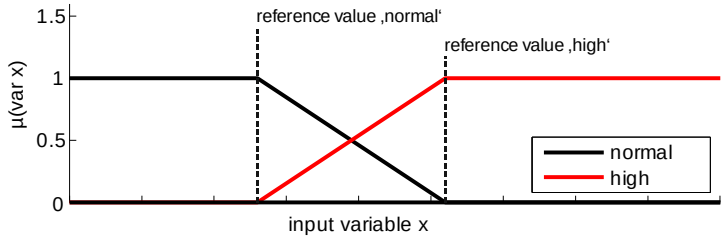


Figure 23: Trapezoid input membership functions. Input variable x with two classes defined by the membership functions 'normal' and 'high'.

2.2.2.2.2. Linguistic rules

Linguistic rules are used to define the relationship between the input and the output variables as shown in Section 1.6. That is, a set of rules are used to define the relationship between the input variables and the output scores in the FIS.

For each input variable a separate output score is calculated. Therefore the following rule structure was used:

Rule1: *IF input variable x IS high* *THEN output score z IS high* **(23)**

Rule2: *IF input variable x IS normal* *THEN output score z IS low* **(24)**

For the calculation of overall scores it is important to connect the input variables and weight their importance for the overall score. For example:

Rule1: *IF input variable x IS high* *THEN output score z IS high* *rule weight: 0.4* **(25)**

Rule2: *IF input variable x IS normal* *THEN output score z IS low* *rule weight: 0.4* **(26)**

Rule3: *IF input variable y IS high* *THEN output score z IS high* *rule weight: 0.6* **(27)**

Rule4: *IF input variable y IS normal* *THEN output score z IS low* *rule weight: 0.6* **(28)**

2.2.2.2.3. Output membership functions

Output membership functions are used for the implication process. Two triangular membership functions 'low score' and 'high score' are defined for the implication step. This definition leads in combination with trapezoid input membership functions to a nearly linear score increase from 0 to 10.

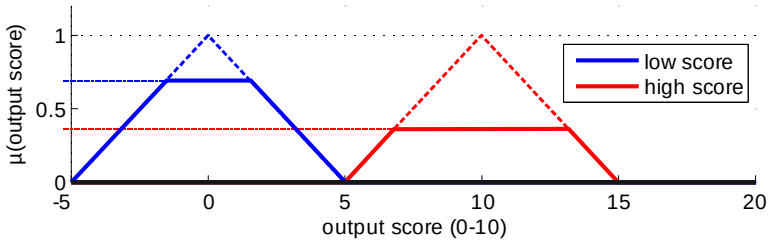


Figure 24: Output membership functions and truncation method. Output score x with two classes defined by the membership functions 'low score' and 'high score'.

Figure 24 shows the triangularly defined output membership functions and the applied 'truncation' method as described in Section 1.6.1.1. The output membership functions are truncated by the adaptability value calculated by the linguistic rules. Defuzzification is done with the 'centroid' method (see (22)).

2.2.2.3. RS-CC-System

Age and gender: The score calculation accounts for age and gender of a subject as shown in the scoring concept (see Section 2.2.1). This is realized by the calculation of adequate reference values for each age group and gender. Therefore, the RS-CC-system uses age and gender of the subject and chooses the age- and gender-related pair of reference value from the pool of reference values. A flow chart of this process is shown in Figure 25. The data for the pool of reference values can be extracted from guidelines or can be calculated based on data of reference cohorts.

Confounder-correction: If a confounder correction is necessary for a variable, the extracted pair of reference values can be shifted to lower or higher values to correct the impact of the confounder. Therefore, a pool of regression equations is used. The pool of regression equations consists of regression equations separated for age and gender. This is important because the relationship between the variable and the confounder can differ between age groups and gender. The RS-CC-system extracts the age- and gender-related regression equation and uses the measured value of the confounder of the subject to shift the reference values to lower or higher values. The measurement of the variable and the confounder has to be done simultaneously because the value of a confounder such as blood pressure can change in a short time period. Additionally, each pair of reference values can be corrected for more than one confounder.

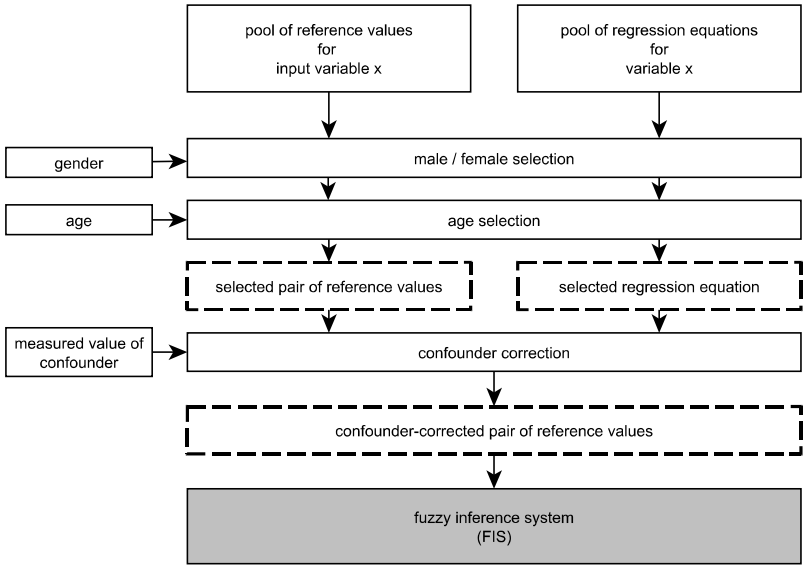


Figure 25: Structure of the RS-CC-system. The system uses two pools of data (pool of reference values and pool of regression equations) for each input variable of the FLSS to select the age- and gender-related pair of reference values and to correct the reference values for the impact of confounders.

Afterwards, the confounder-corrected pair of reference values is used in the FIS to generate the membership functions ‘normal’ and ‘high’. In conclusion, the shift of reference values leads to a shift of membership functions as shown in Figure 26.

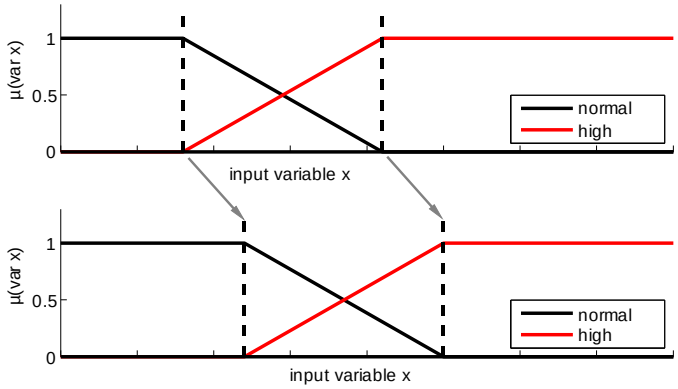


Figure 26: Shift of membership functions – example. The membership functions ‘normal’ and ‘high’ (top graphic) are shifted to higher values (bottom graphic) for the impact of a confounder.

2.2.2.3.1. Multiple linear regression model

A multiple linear regression model was used in the RS-CC-System to shift the pair of reference values and respectively the membership functions 'normal' and 'high' to lower or higher values to correct the impact of confounders. However, it is possible to use different regression models in the RS-CC-System.

Multiple linear regression model: The following equation (see (29), found and adapted from Backhaus et al., 2008, p64) shows the used multiple linear regression equation. This equation estimates a linear dependency between a dependent variable (Y) and an independent variable (X). For every independent variable a linear term a_jX_j can be added to the equation. The constants a_j are the regression coefficients.

$$\hat{Y} = a_0 + a_1X_1 + a_2X_2 + \cdots + a_jX_j + a_JX_J \quad \textbf{(29)}$$

$\hat{Y}$ = estimation of dependent variable Y
$a_0, a_1, \ldots, a_n$ = regression coefficients
X = independent variable

Parameter identification process: A parameter identification process can be used to estimate the regression coefficients. Therefore the following objective function (see (30), found and adapted from Backhaus et al., 2008, p64) can be applied. The target is to minimize the sum of the squares of the differences between the dependent variable and the estimated dependent variable. This process results the regression coefficients that shows the best fit.

Objective function:

$$\sum_{k=1}^{K} e_k^2 = \sum_{k=1}^{K} [y_k - (a_0 + a_1x_{1k} + a_2x_{2k} + \cdots + a_jx_{jk} + \cdots a_Jx_{Jk})]^2 \rightarrow min \quad \textbf{(30)}$$

e_k = residual values (k = 1, 2, …, K)
y_k = values of dependent variable (k = 1, 2, …, K)
a_0 = constant term
a_j = regression coefficients (j = 1, 2, …, J)
x_{jk} = values of independent variables (j = 1, 2, …, J; k = 1, 2, …, K)
J = number of independent variables
K = number of observations

2.2.2.3.2. Confounder correction process

The age- and gender-related multiple linear regression equation is extracted from the pool of regression equations and used to shift the pair of reference values and respectively the membership functions 'normal' and 'high' to lower or higher values to correct the impact of confounders.

Therefore, a shift (Δr) was calculated based on the multiple linear regression equation and was added to the reference values (r_{normal} and r_{high}). In conclusion, the confounder corrected reference values were calculated as:

$$r^*_{normal} = r_{normal} + \Delta r \quad \textbf{(31)}$$

$$r^*_{high} = r_{high} + \Delta r \quad \textbf{(32)}$$

The shift is calculated based on the extracted multiple linear regression equation. Reference values for each confounder (b_j) have to be defined. However, the shift is calculated as the difference of the result of the regression equation calculated by the measured value of each confounder (c_j) of the subject and the result of the regression equation calculated by the reference value of each confounder (see (33)).

$$\Delta r = \left(a_0 + a_1 c_1 + a_2 c_2 + \cdots + a_j c_j + \cdots a_J c_J\right)\ldots \quad \textbf{(33)}$$
$$-\left(a_0 + a_1 b_1 + a_2 b_2 + \cdots + a_j b_j + \cdots a_J b_J\right)$$

2.3. Results

An example of the resulted behavior of the FLSS for an input variable x is shown in Figure 27. This figure shows a linear increasing score ramp calculated by the FLSS for four different age groups. The score ramp is shifted to higher values for each decade of age. Furthermore, each score ramp is shifted to lower or higher values for the impact of confounders.

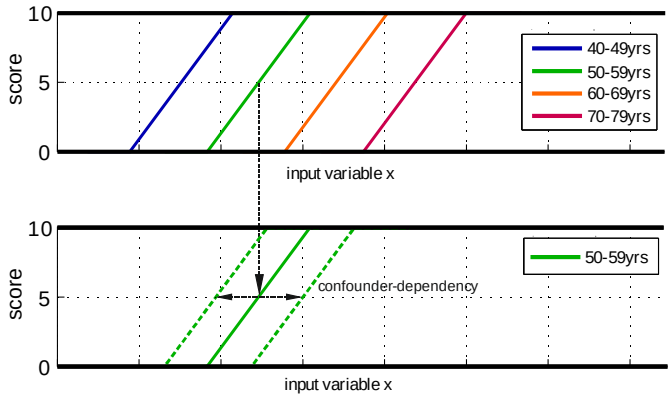


Figure 27: Example: score ramp calculated by the FLSS. The top graphic shows four linear increasing score ramps for four different age groups. The bottom graphic shows the shift of a score ramp for the impact of a confounder.

However, calculated score ramps are theoretical linear but the calculation dynamic of fuzzy logic leads to a smooth sigmoidal increase. Figure 28 shows the score ramp calculated by the FLSS.

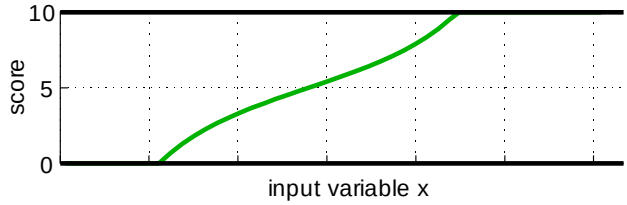


Figure 28: Sigmoidal score ramp. Calculated score ramps are sigmoidal due to the calculation dynamic of fuzzy logic.

2.4. Discussion

2.4.1. Interpretation of score results

Cardiovascular prevention plays an important role due to the reduction of the economic and human burden of CVD. Scores can provide additional information on the health status of the arterial system or the individual risk of a patient. However, the advantage of a score is the clear output (score from 0 to 10) – conceptually easy to understand for patients. However, established scores for the cardiovascular system differ in their methodology and informative content.

Most established scores for the cardiovascular system are risk scores based on scoring schemes. For instance, the Framingham risk score estimates the 10-year absolute risk of CHD events (NCEP, 2002). A similar approach was presented as PROCAM score (Assmann et al., 2002). Conroy et al. (2003) or D'Agostino et al. (2008) presented general CVD risk scores. Some scores give additional information about the heart age such as the PROCAM Score (Assmann et al., 2002) or the SCORE project (Cuende et al., 2010). However, these score schemes are based on statistical methods such as the Cox proportional-hazard regression model. Therefore, data of follow-ups of prospective cohort studies are used. Commonly used variables for the risk assessment are: age, gender, smoking, SBP, diabetes mellitus, medication status, TC, LDL cholesterol and HDL cholesterol.

However, established scores are black-box models which use a fundamental different mythology compared to the FLSS. Therefore, it is important to note that output scores calculated by the FLSS can give information about abnormal high or low values compared to normal values for the same age group and further about the severity of the change from normal values to abnormal values. Hence, the FLSS can give no information about the individual risk of a subject. This is a fundamental difference between scores calculated by the FLSS and established risk scores. In conclusion, the FLSS serves cross-sectional information and risk scores serves longitudinal information as shown in Figure 29.

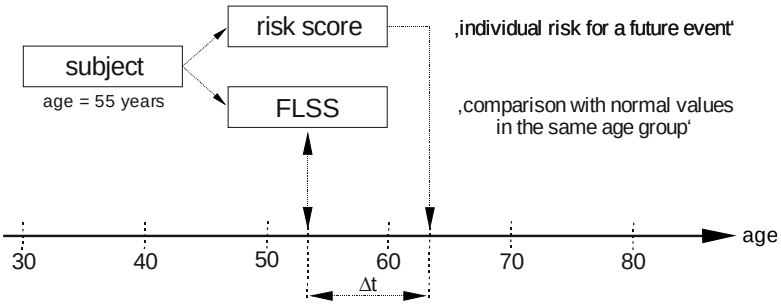


Figure 29: Risk scores versus output scores of the FLSS. Risk scores serve longitudinal information about the individual risk of a subject for having a cardiovascular event in a defined time period (Δt). Contrary, scores calculated by the FLSS serves information about the relative change of a variable from normal values to abnormal values at the time of measurement.

2.4.2. Normal, reference and cut-off values

Reference values and normal values are important for the clinical use of a measurement. The assessment of the severity of a pathophysiological change is important to assess how much a variable has changed compared to the normal level. It follows, that single cut-off values are not satisfactory because they serve only information about the normality but no information about the severity of a change from normal values to abnormal values. Therefore, guidelines (e. g. WHO hypertension guidelines (Chalmers et al., 1999), JNC7 hypertension guidelines (Chobanian et al., 2003), ESH/ESC hypertension guidelines (Mancia et al., 2007)) define different classes of blood pressure to classify a measured blood pressure value into normal or hypertensive categories. Unfortunately, the definition of adequate normal and reference values is still left for a variety of indicators and markers. In conclusion, it is mandatory for the FLSS to use reference values which serve information about the normality of a variable and the severity of the change of a variable from normal to abnormal values.

2.4.3. Scoring concept

Usable indicators or markers for the FLSS: The presented scoring concept is an approach to give information about the cardiovascular health status and further to account for the physiological change of an indicator or variable with age. Therefore, it is important to differentiate between the physiological course and the pathophysiological course of a variable with age as basically described in the concept of normal and early vascular aging (see Section 1.5.2). For instance, a pathophysiological increase of an indicator or marker can be the result of structural changes of heart or vessels (e.g. increase of arterial stiffness) due to the long-term impact of risk factors (e.g. smoking, physical inactivity or diabetes mellitus) or genetic predisposition.

Hence, only indicators or markers which give information about the physiological or pathophysiological status of heart or vessels are useable for the FLSS (e.g. PWV, intima media thickness or flow mediated dilatation). In contrast, blood indicators (e.g. glucose, total cholesterol, LDL cholesterol or HDL cholesterol) are useful to estimate long-term risk but they cannot reflect the present health status of heart or vessels.

Score results: The scoring concept uses reference values in decades of age. It follows, that there is a tendency of higher scores for younger subjects and lower scores for older subjects in each age group. However, an advantage of an age-dependent score is that it can be positively influenced by lifestyle changes with age. That means if a subject has a high score at the age of 45 a change of lifestyle could lead to a lower score at the age of 55.

2.4.4. Methodology of the FLSS

Fuzzy logic is used for a variety of applications in the research field of cardiovascular systems, cardiovascular risk estimation or medical decision making. For instance, a decision making system to perform prognostic modeling of disease states (Anderson et al., 2000), a fuzzy logic system to assess the cardiovascular autonomic function (Carvalho et al., 2002), a fuzzy system to measure the global cardiovascular risk based on risk factors associated with CVD and stroke (Chan and Benzie, 2010), a

risk classification by fuzzy inference (Horgby, 1998), a fuzzy decision support system for therapy administration in cardiovascular intensive care patients (Denai et al., 2007).

The FLSS is based on an extended FIS (see Section 1.6.1.1). The FIS is used to map a real value to an abstract score. Additional components (RS-CC-system) are used to adjust the mapping process for age, gender and the impact of confounders. Therefore the FLSS abstracts a real value to an abstracted value (0-10) as shown in Figure 30. In conclusion, the FLSS is a multivariable approach and uses external data in terms of reference values and regression lines for the mapping process. An important advantage of a scoring system based on a FIS is that the relationship between input variable and score can be understood by the user and the system considers the impact of age and confounders.

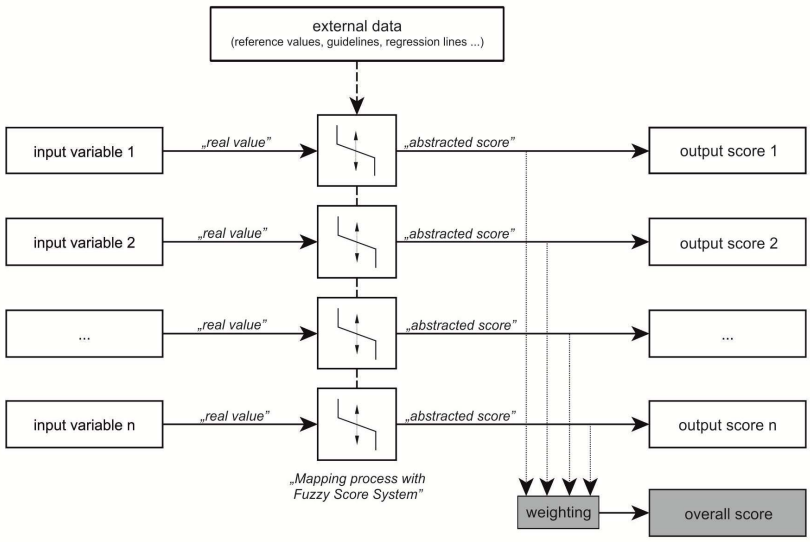


Figure 30: Mapping process of the FLSS. Real values are mapped by the FLSS to abstracted scores (0-10). The mapping process is defined by external data and can be adjusted for age, gender and confounders.

External data: The FLSS serves a method to calculate scores based on external data (reference values, data from guidelines or regression equations). Therefore it is important to note that the accuracy of the score results is predominantly defined by the accuracy of the external data. Hence, the use of adequate reference values and regression lines is of particular importance for the score calculation.

Shape of score ramp: The nearly linear increasing score from 0 to 10 is an important simplification of this approach. However, the FLSS is able to use different shapes of membership functions and therefore it is possible to generate different shapes for the score ramp. Although there is no evidence which shapes are close to reality. Additionally, the score ramp increases theoretical linear but the calculation dynamic of fuzzy logic leads to a smooth sigmoidal increase of scores (see Figure 28). In conclusion, it is clear that further investigations are important to estimate which score ramp shape would be adequate for a specific input variable.

Confounder-correction: Most relationships between input variables and confounders are non-linear. Therefore the used linear regression model is a further simplification of the FLSS. Linear regression is only a good approximation of the confounder-dependency in a small interval of values. Therefore, the impact of this simplification has to be estimated for each input variable and confounder.

2.4.5. Usefulness of fuzzy logic

This work has shown that fuzzy logic has an additional benefit for the implementation of the scoring concept due to the advantage that fuzzy logic is directly understandable and interpretable by the user and the change from normal to abnormal values of an indicator or marker can be described by fuzzy sets. Therefore, fuzzy logic provides an approach to define the individual state of an indicator or marker as smooth transition between a healthy state and a diseased state instead of sharp cut-off values. In addition, fuzzy logic can be easily extended and adapted to implement the confounder-correction for confounders of the measurement by shifting of membership functions.

2.4.6. Relevance for clinical practice

A scoring system could be an additional alternative to provide information on the cardiovascular health status of heart and arteries. An advantage of a scoring system is the clear output (score from 0 to 10) – conceptually easy to understand for patients. Furthermore, a scoring system can consider age and confounders. Sometimes it can be too complicated and time-consuming for preventive examinations to account for the impact of age and confounders. However, scores are only additional tools to screen subjects for their cardiovascular health status but they cannot replace a clinical examination by experts.

2.5. Conclusions

The scoring concept and the implementation of the scoring concept based on fuzzy logic (FLSS) is a novel approach to assess the individual cardiovascular health status in relation to normal and reference values of a healthy group of subjects. Based on the scoring concept, it is possible to account for the physiological aging of the cardiovascular system and to account for the effect of gender, and confounders of the measurement. In contrast to other score systems for risk stratification, the present score system gives cross-sectional information on the normality or abnormality of an indicator or marker at the time of measurement. A further advantage of the present scoring technique is that calculated scores can be easily interpreted by patients and this technique can also be implemented into devices.

However, the correctness of calculated score results is predominantly dependent on the external data such as reference values and regression equations. Furthermore, the definition of the shape of the score ramp and the use of different regression models (linear regression model or non-linear regression model) has significant impact on the score calculation. Therefore, it is important to obtain sufficient and validated external data and to investigate an adequate regression model and an adequate shape of the score ramp for each indicator or marker.

3. Setup for surrogate variables of arterial stiffness

3.1. Introduction

The FLSS was developed in an adaptable manner and can be used for different indicators or markers. However, for this investigation the FLSS was setup for surrogate variables of arterial stiffness. This was done to investigate the use of arterial stiffness to assess the structural change of arterial vessels expressed as pathophysiological increase of arterial stiffness in relation to normal values. Details can be found in Section 1.7.3.

Therefore this chapter describes the setup process of the FLSS for surrogate variables of arterial stiffness: pPP, cPP, PWV and cAI. As described in Chapter 2, it is necessary for the practical setup of the FLSS to extract external data (reference values and regression lines). However, due to the lack of reference values for surrogate variable of arterial stiffness, external data was calculated based on data of the ACCT database.

3.1.1. Surrogate variables of arterial stiffness

Various surrogate variables including pPP, cPP, PWV and cAI index can be non-invasively measured to assess arterial stiffness (Laurent et al., 2006). PWV is essentially a 'direct measurement' of functional arterial stiffness (Laurent et al., 2006), whereas pPP, cPP and AI are 'indirect measurements', since they are also affected by wave propagation in addition to elasticity changes in the arterial wall (Nichols and O'Rourke, 1998, p201-222).

3.1.1.1. Pulse wave velocity

The measurement of PWV can be seen as a 'direct measurement' of arterial stiffness and it is generally accepted as the most simple, robust and reproducible method (Laurent et al., 2006). Further it is included in the 2007 ESH/ESC guidelines for the management of hypertension (Mancia et al., 2007).

The relationship between PWV and arterial stiffness is described by the Moens-Korteweg equation (see (34)) and is derived from Newton's second law of motion $F = m \cdot a$ (Nichols and O'Rourke, 1998, p63). This equation shows that PWV is dependent on the elasticity of the vessel wall (incremental elasticity modulus (E_{inc})), the wall thickness (h), the vessel radius (r) and the density of the blood (ρ).

$$PWV = \sqrt{\frac{E_{inc}\, h}{2\, r\, \rho}} \qquad \textbf{(34)}$$

Measurement of PWV with pressure-waveforms: The gold-standard of PWV measurement is the carotid-femoral method (Laurent et al., 2006). In this technique, a pressure waveform is measured by

arterial tonometry or pulse detection devices at the common carotid artery and at the femoral artery. PWV is defined as the distance (Δx) travelled by the waveform divided by the time (Δt) for the wave to travel this distance, as shown in following equation.

$$\mathrm{PWV} = \frac{\Delta x}{\Delta t} \qquad \textbf{(35)}$$

Foot-to-foot velocity method: This is the common methodology to assess the transit time (Δt) which is calculated as the foot-to-foot time difference (Δt) between the foot of the common carotid artery waveform and the femoral artery waveform. The distance (Δx) is measured on the surface of the body.

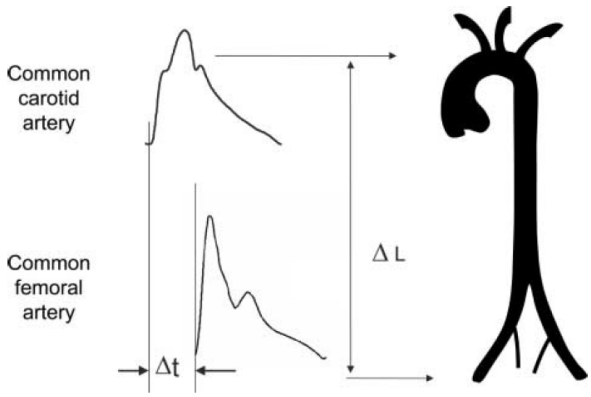


Figure 31: Foot-to-foot velocity method. (reproduced from Laurent et al., 2006).

Relevance of PWV as indicator for CVD risk and mortality: PWV is strongly associated with cardiovascular events and all-cause mortality: Blacher et al. (1999a,b), Laurent et al. (2001), Cruickshank et al. (2002), Boutouyrie et al. (2002), Mattace-Raso et al. (2006), Willum-Hansen et al. (2006), Mitchell et al. (2010), Vlachopoulos et al. (2010).

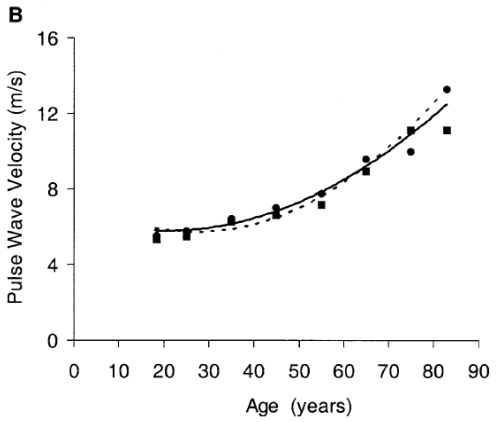


Figure 32: PWV and age: for males (circles, solid lines) and females (squares, dashed lines) (reproduced from McEniery et al., 2005).

PWV and age: McEniery et al. (2005) showed a non-linear increase of PWV with age for male and female subjects (healthy and normotensive population (4001 subjects)). Further it was hypothesized that PWV is a more sensitive marker in subjects after the age of 50. Figure 32 shows the increase of PWV with age.

PWV and gender: McEniery et al. (2005) observed no significant differences in aortic or brachial PWV between men and women.

3.1.1.2. Pulse wave transmission and reflection

The measured central aortic pressure waveform (P_m) is a supercomposition of a forward travelling wave (incident wave – P_i) and a reflected wave from the periphery (P_r) (Nichols and Singh, 2002) (see (36)). That is, for each heart cycle the left-ventricle ejects a forward travelling wave and this wave is reflected by impedance mismatch such as arterial branching, changes in arterial diameter and changes in vessel wall material stiffness (Butlin, 2007).

$$P_m = P_i + P_r \qquad \textbf{(36)}$$

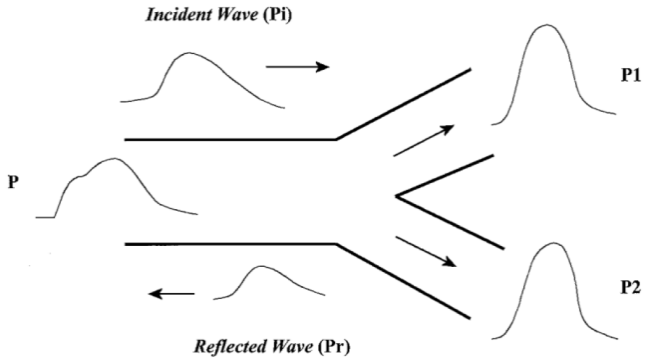


Figure 33: Composition principle of the central aortic pressure waveform. The central aortic pressure waveform is a superposition of both a forward wave and a reflected wave. (reproduced from Hirata et al., 2006)

With an increase in arterial stiffness the transmission velocity of both forward and reflected wave increases (Nichols and Singh, 2002). The increase of transmission velocity leads to an earlier arrival of the reflected wave in the central aorta and augments pressure in the late systole. This augmentation leads to an increase in central aortic pressure. Hence, an increased cPP is an 'indirect measure' of an increase in arterial stiffness (Laurent et al., 2006).

$$P_i = \frac{(P_m + Z_C \cdot Q_m)}{2} \qquad \textbf{(37)}$$

$$P_r = \frac{(P_m - Z_C \cdot Q_m)}{2} \qquad \textbf{(38)}$$

$$Q_i = \frac{(Q_m + \frac{P_m}{Z_C})}{2} \qquad \textbf{(39)}$$

$$Q_r = \frac{(Q_m - \frac{P_m}{Z_C})}{2} \qquad \textbf{(40)}$$

Decomposition of the incident and reflected wave: It is possible to decompose the incident and the reflected waveform from the measured pressure waveform. The simultaneous measurement of pressure (P) and flow (Q) at the same site results in a frequency-dependent impedance spectrum, characteristic impedance (Z_c) may be derived and equations (37)-(40) solved to yield the incident and reflected pressure and flow waves (Nichols and O'Rourke, 1998, p214).

Figure 34 shows the measured pressure and the measured flow in the ascending aorta of a middle-aged patient. The dashed line indicates the decomposed incident wave ejected from the left-ventricle. It is shown how the measured pressure waveform is augmented by wave reflections.

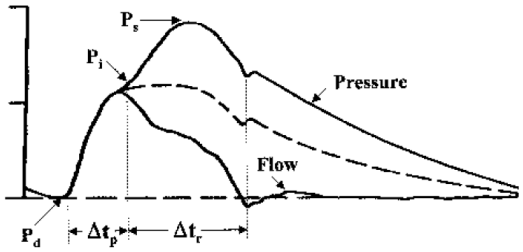


Figure 34: Decomposition of a measured aortic pressure waveform. The figure shows the measured pressure and the measured flow in the ascending aorta of a middle-aged patient and the decomposed incident pressure waveform (dashed curve). (reproduced from Nichols and Singh, 2002)

This figure shows further the systolic blood pressure (p_s), the diastolic blood pressure (p_d) and the inflection point (p_i). The inflection point indicates the beginning of augmentation of the pressure waveform due to wave reflections and can be used to estimate the pressure augmentation without decomposition of the pressure waveform. Therefore, the augmentation pressure is measured as p_s minus p_i and the AI is defined as the augmentation pressure related to the PP (systolic blood pressure minus diastolic blood pressure). See (41) (found in Nichols and O'Rourke, 1998, p215).

$$AI = \frac{(p_s - p_i)}{(p_s - p_d)} \qquad \textbf{(41)}$$

If the inflection point occurs after the systolic peak, the AI is negative and defined as shown in (42) (found in Nichols and O'Rourke, 1998, p215).

$$AI = \frac{(p_i - p_s)}{(p_s - p_d)} \qquad \textbf{(42)}$$

The AI is not only an indicator for the central aortic pressure augmentation and can be used as well to assess the peripheral pressure augmentation. Therefore, AI can be defined as shown in (43) (found in Millasseau et al., 2003).

$$AI = \frac{(p_i - p_d)}{(p_s - p_d)} \qquad \textbf{(43)}$$

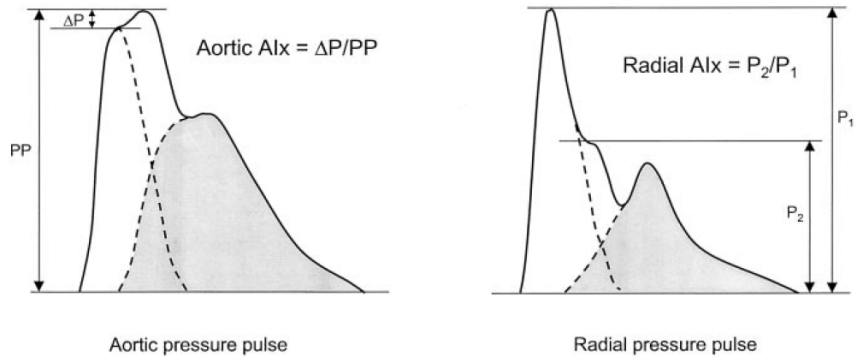


Figure 35: Aortic AI versus radial AI. The adequate definition of the AI is depending on the waveform. (reproduced from Millasseau, 2003)

Relevance of AI as indicator for CVD risk or mortality: Several studies indicate that AI may be a useful marker of cardiovascular risk and cardiovascular mortality, or is increased in diseased patients (Wilkinson et al. (2000), Nürnberger et al. (2002), Chirinos et al. (2005), Payne et al. (2007)). Zoungas and Asmar (2007) suggested that AI may independently predict all-cause mortality and cardiovascular events in coronary and end-stage renal disease patients, but some outcome studies have questioned its usefulness in hypertensive subjects and dialysis patients.

AI and age: AI increases steadily with age but reaches a plateau after the age of 50 years (McEniery et al., 2005). Fantin et al. (2007) showed similar results. Therefore, McEniery et al. (2005) hypothesized that AI is a better predictor in younger and PWV in older subjects because PWV increases predominantly in older subjects. Figure 36 shows AI and augmentation pressure with age for male and female subjects.

AI and gender: McEniery et al. (2005) showed that AI is higher for female than for male subjects over the whole age range. Similar results were presented by Fantin et al. (2007) and Janner et al. (2009).

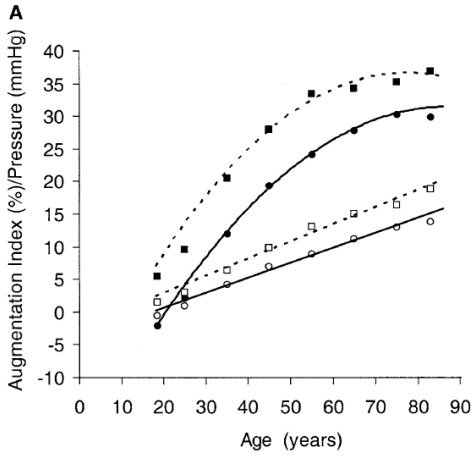


Figure 36: AI and augmentation pressure with age: for a healthy and normotensive population (4001 subjects). Lines are drawn for males (circles, solid lines) and females (squares, dashed lines). Open circles and open squares indicate augmentation pressure. Closed circles and closed squares indicate AI. (reproduced from McEniery et al., 2005).

3.1.1.3. Central and peripheral blood pressure

Central and peripheral pulsatile blood pressure differs in shape and absolute values along the arterial tree. Therefore, central and peripheral blood pressure cannot be used interchangeable as marker. The change in wave form and pressure amplitudes results from an impedance change. That is, the pressure amplitude increases along the arterial tree due to an increase of low frequency components of the impedance and the change of the various frequency components in terms of their distance from the main reflection site leads to a change in the pressure wave form (Nichols and O'Rourke, 1998, p171).

Figure 37 shows the amplification and the change in wave form along the arterial tree from the central aorta to peripheral vessels. It is shown that PP calculated as SBP minus DBP increases markedly. A position statement by Laurent et al. (2006) concluded that it is inaccurate to use brachial PP as a surrogate for aortic or carotid PP due to the pressure amplification between central and peripheral arteries, particularly in young subjects.

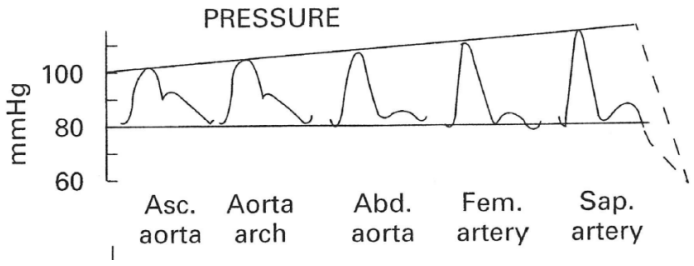


Figure 37: Pressure amplification along the arterial tree. PP increases with increasing distance from the heart. (reproduced from Nichols and O'Rourke, 1998, p171)

pPP and cPP as indicators for CVD risk: cPP and pPP are used as markers for an increase in arterial stiffness and cardiovascular risk. However, cPP and pPP cannot be used interchangeably due to different interactions with elasticity changes in the arterial tree. It is known that DBP differs only slightly between central and peripheral sites but SBP is influenced differently between these two sites. The peripheral SBP is predominantly influenced by elasticity changes of the arterial wall and the central SBP due to the elasticity changes of the arterial wall and an earlier arrive of the reflected wave (McEniery et al., 2005). Franklin et al. (1999) concluded that higher pPP was an important component of risk and neither peripheral SBP nor peripheral DBP was superior to pPP in predicting CHD. Furthermore, there is some evidence that central blood pressure is more related to cardiovascular events or atherosclerosis than brachial blood pressure: Safar et al. (2002), Williams et al. (2006) and Roman et al. (2007).

pPP and age: Franklin et al. (1997) showed that pPP increases predominantly after the age of 50 years. This study also showed that after the sixth decade of age an increase in pPP and a decrease in DBP is a surrogate measurement for large artery stiffness. Figure 38 shows how blood pressure changes with age for different blood pressure categories. This figure shows an increase of SBP with age whereas DBP increases for younger and decreases for older subjects.

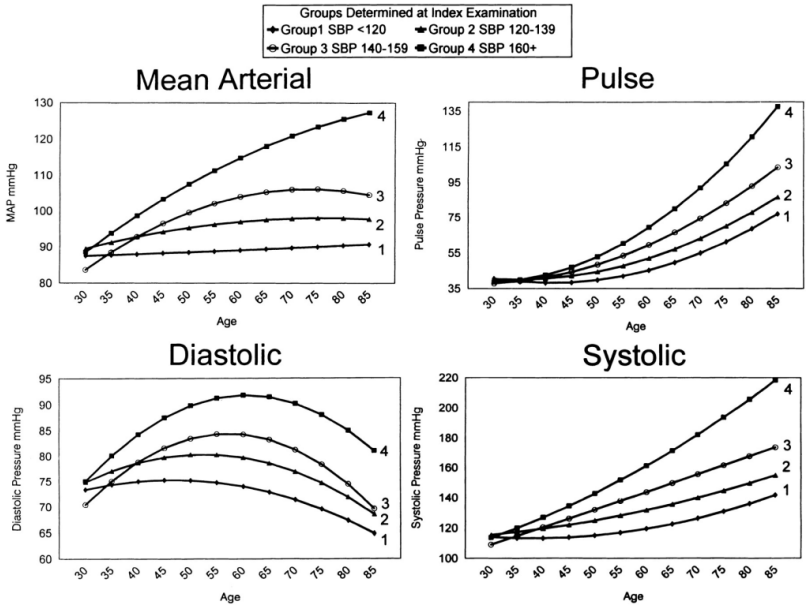


Figure 38: Regression lines of peripheral blood pressure and age: categorized for four different blood pressure categories. (reproduced from Franklin et al., 1997)

cPP and age: Figure 39 shows the change of cPP with age. It is shown that the increase of cPP for middle-aged subjects is predominantly driven by earlier and higher wave reflections. Results of McEniery et al. (2005) indicate that cPP increases linearly with age due to the linear increase of augmentation pressure which is largely driven by increased wave reflections (McEniery, 2005).

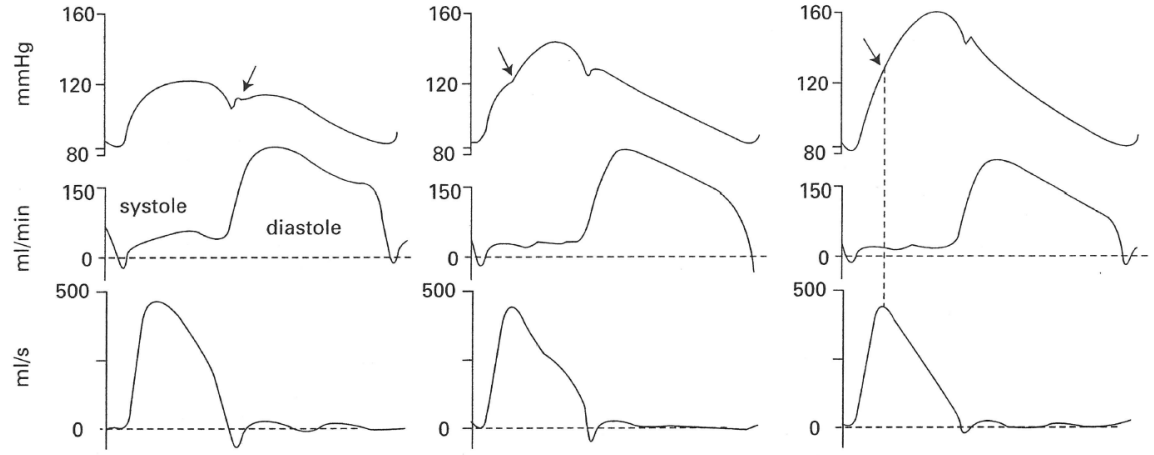


Figure 39: Pressure and flow in the ascending aorta and in the coronary aorta (normotensive subjects). This figure shows the pressure in the ascending aorta (top) and the pressure in the coronary aorta (middle) for adolescent subjects (left), middle- aged subjects (middle) and elderly subjects (right). (reproduced from Nichols and O'Rourke, 1998, p217)

Pulse pressure amplification and age: The PP increases from central to peripheral sites as shown in Figure 37. It is known that the PP amplification (the ratio of pPP to cPP) decreases with age because of a proportional higher increase in cPP (McEniery et al., 2005).

cPP and pPP, and gender: McEniery et al. (2005) showed that the widening of pPP is more prominent in women than in men and central SBP increased more prominent in women than in men. This study also indicated that the PP amplification is significantly higher for male than female subjects.

3.1.1.3.1. Measurement of central blood pressure

Peripheral blood pressure can be measured easily and non-invasively with standard measuring methods (palpatory, auscultatory and oscillometric sphygmomanometry). In contrast, the non-invasive measurement of central blood pressure is much more difficult and can be performed by different methodologies:

Non-invasive estimation of central aortic pressure using a transfer function: Transfer functions were developed and investigated to calculate the aortic pressure waveform from the radial artery pressure waveform as shown in studies by Karamanoglu et al. (1993), Chen et al. (1997), Cameron et al. (1998), Pauca et al. (2001) and Sharman et al. (2006). This mathematical technique is used in several commercial measurement devices.

Non-invasive estimation of central aortic pressure using carotid tonometry: The central aortic AI can be estimated non-invasively by using carotid artery tonometry. Chen et al. (1996) validated this technique compared to invasive diagnostic catheterization for the estimation of AI of the ascending aortic pressure under various physiological conditions. This study showed that carotid AI is highly correlated with central aortic AI. The carotid artery waveform can be calibrated for the brachial mean and diastolic pressures as described by Safar et al. (2002). This approach can be used to estimate central SBP, central DBP and respectively cPP with carotid tonometry because of the small degree of pressure amplification between the aorta and the carotid artery.

3.1.2. Impact of confounders

The measurement of surrogate variables of arterial stiffness depends on various confounders. Therefore it is essential to estimate the impact of a confounder on the measured result at the time of a measurement. This section describes the relationship of the main confounders of PWV and AI.

3.1.2.1. Confounders of PWV

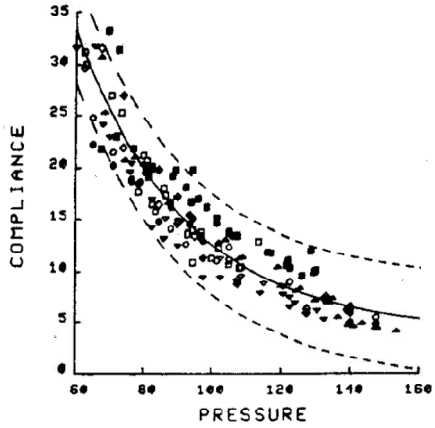


Figure 40: Decrease of arterial compliance as a function of blood pressure: obtained in the femoral artery. (published by Megerman et al. (1986); figure was extracted from Li (2000, p57))

PWV and blood pressure: The elasticity and respectively the compliance of an arterial vessel are related to the level of distending blood pressure. As shown by Megerman et al. (1986), with increasing pressure levels the compliance of the femoral artery decreases. It follows, based on the Moens-Korteweg equation (see (34)), that a change in arterial elasticity leads to a change in PWV. In conclusion, blood pressure is related to PWV. Carroll et al. (1991) investigated this relationship for humans in different age categories. Figure 41 shows regression lines calculated for PWV and DBP. This figure also shows that the relationship between PWV and blood pressure becomes steeper with increasing age due to a decrease in arterial distensibility.

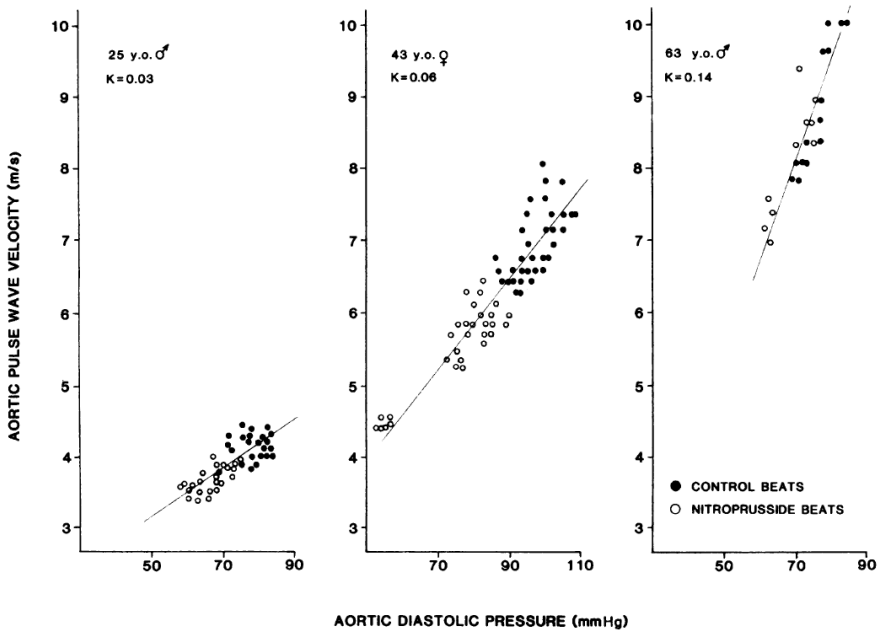


Figure 41: Relationship between aortic PWV and aortic DBP for different ages. 'The relationship between pulse wave velocity and aortic diastolic pressure is shown for three patients of different ages but all with dilated cardiomyopathy. Note that as pressure was lowered by nitroprusside there was a fall in pulse wave velocity in all patients, but the relationship was much steeper (increase slope, K) for the oldest patient.' (Carroll et al., 1991).

PWV and HR: The work of Lantelme et al. (2002) showed that PWV changes during cardiac pacing at different pacing frequencies (60, 70, 80, 90, 100 bpm) although, no significant blood pressure variations were observed. The conclusion of this study was that HR is an important factor in the intra-individual variation of PWV in elderly subjects, essentially independent of blood pressure effects.

3.1.2.2. Confounders of AI

AI and HR: Studies indicate that AI is affected by HR. A study from Wilkinson et al. (2000) measured 22 subjects with permanent cardiac peacemakers. This study demonstrated an inverse and linear relationship between cAI and HR (for a 10 beats/min increment, AI fell around 4%). Figure 42 shows this relationship. Wilkinson et al. (2000) hypothesized that a change in HR alters the relative timing of the return of the reflected wave to the ascending aorta and this leads to an inverse and linear relationship between cAI and HR. A study of Gatzka et al. (2001) estimated the confounding effect of HR on cAI in elderly essential hypertensive (873 subjects) under using of carotid artery tonometry and

simultaneous continuous wave Doppler measurement of the ascending aortic blood flow. The results of this work provided a regression equation to correct the impact of HR on AI.

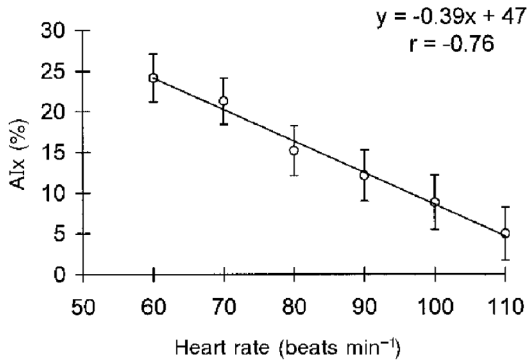


Figure 42: Linear regression between cAI and HR: This figure shows a linear decrease of cAI for an increase in HR (reproduced from Wilkinson et al., 2000).

AI and body height: Studies by Marchais et al. (1993), Yasmin and Brown (1999) and McGrath et al. (2001) indicate that there is an inverse relationship between AI and body height. McGrath et al. (2001) concluded that body height is a key determinant of arterial wave reflections (AI) and further that body height accounts largely for the gender differences in AI results.

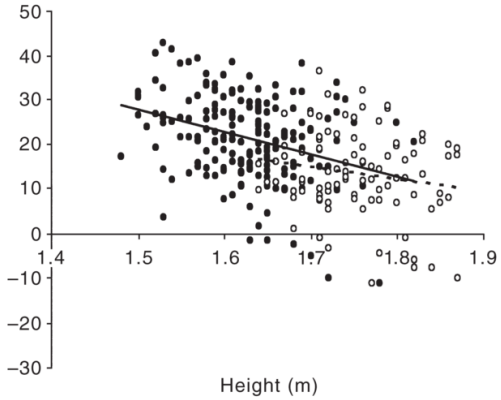


Figure 43: Linear regression between AI and body height: in men (○) and in women (●). This figure shows a linear decrease of AI (vertical axis) for an increase in body height (reproduced from McGrath et al., 2001).

3.1.3. Normal and reference values

In general terms, normal and reference values define different categories. Reference values reflect the mean, median or the range of values in a specific population. In contrast, normal values define the normal level of a variable. Therefore, reference values which are calculated in a group of 'healthy' subjects without any risk factors or diseases can be used to define normal values. In addition, normal values can be defined on morbidity or mortality data so that the individual risk is low. In addition to this approach, the scoring concept of the FLSS (see Section 2.2.1) needs to define an interval of 'normal', 'elevated' and 'high' values for each variable and age category. Hence, the FLSS needs a

pair of reference values ‘normal’ and ‘high’ for each variable and age category. Therefore, this set of reference values includes information of the normality and of the severity of the change of a variable from normal to abnormal values.

Some studies and guidelines have presented reference values or defined normal values. Normal and reference values can differ between studies depending on the selected reference cohort, the used measurement methodology, calculation procedures and devices. Following reference values, normal values and guidelines are presented for pPP, cPP, PWV and AI in the literature.

3.1.3.1. PWV reference values

The 2007 ESH/ESC hypertension guidelines (Mancia et al., 2007) proposed a threshold value of 12 m/s for aortic PWV. This threshold was defined as fixed value and doesn’t take factors such as age and blood pressure into account. Alecu et al. (2008) presented reference values for elderly subjects (60-75 years), whereas an aortic PWV below 10 m/s can be considered as ‘normal’, between 10 to 13 m/s as ‘high normal’ or ‘borderline’ and above 13 m/s as ‘elevated’. A further approach was presented by McEniery et al. (2005). Results of this approach are shown in Figure 32. Mattace-Raso et al. (2010) presented normal (1455 subjects) and reference values (11 092 subjects) for carotid-femoral PWV calculated on a large European population. Furthermore, this study analyzed the difference of PWV values according to different blood pressure categories. Figure 44 shows the calculated PWV reference values for different age and blood pressure categories. It shows how PWV increases with age and further how PWV depends strongly on blood pressure. Therefore, PWV is much higher in a group of hypertensive subjects compared to normotensive subjects in the same age category.

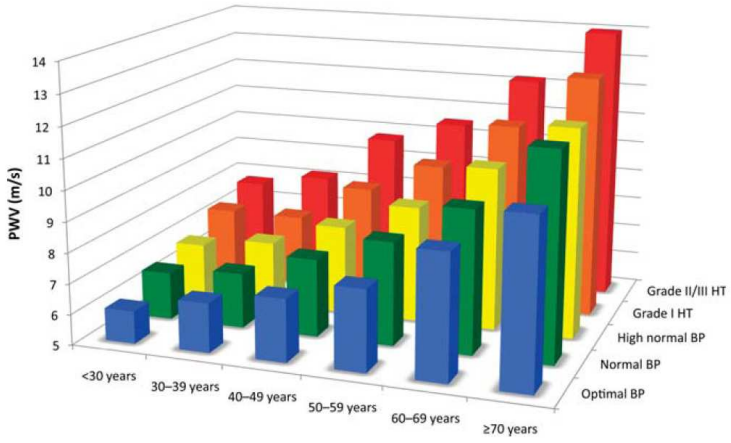


Figure 44: PWV reference values calculated on a large European cohort: categorized for age and blood pressure levels (reproduced from Mattace-Raso et al., 2010).

Figure 45 shows the calculated normal values for PWV by Mattace-Raso et al. (2010). Therefore, a normotensive group without overt CVD, current treatment, diabetes mellitus and risk factors were extracted (Mattace-Raso et al., 2010). It is shown that the median of PWV increases from 6.1 m/s for young subjects (<30 years) to 10.6 m/s (≥70 years) for old subjects.

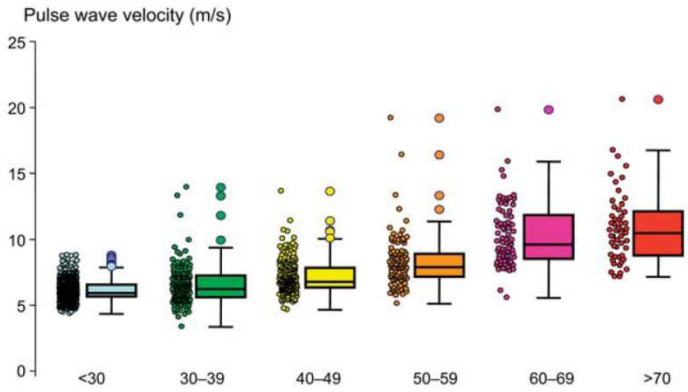


Figure 45: PWV normal values calculated on a large European cohort: categorized for age (reproduced from Mattace-Raso et al., 2010).

3.1.3.2. AI reference values

The progress of definition of normal and reference values for AI is not as advanced as PWV. McEniery et al. (2005) presented reference values for central and peripheral AI based on a cohort of 4001 healthy and normotensive individuals as shown in Figure 36. In contrast, Janner et al. (2009) presented reference values for cAI in a large unselected population (4561 individuals). This study found the same curve linear increase of AI with age as McEniery et al. (2005).

3.1.3.3. pPP reference values

PP is defined as the difference between SBP and DBP. A variety of hypertension guidelines (e.g. WHO hypertension guidelines (Chalmers et al., 1999), JNC7 hypertension guidelines (Chobanian et al., 2003), ESH/ESC hypertension guidelines (Mancia et al., 2007)) are available that define thresholds or categories for peripheral SBP and peripheral DBP. Most of these guidelines define a peripheral SBP $\geq$ 140 in combination with a peripheral DBP < 90 mmHg as isolated systolic hypertension which is a pPP > 50 mmHg. ESH/ESC hypertension guidelines (Mancia et al., 2007) concluded that while values such as 50 or 55 mmHg have been suggested, no practical cutoff values separating pPP normality from abnormality at different ages were produced and that pPP may be used to identify elderly patients with systolic hypertension who are at a particularly high risk. Asmar et al. (2001) presented reference values for clinic brachial PP in a nonselected population. It was shown that 50 mmHg is likely the reference value for clinic PP in both men and women.

3.1.3.4. cPP reference values

There is still a lack of normal values for cPP. A consensus document on central blood pressure measurement (Agabiti-Rosei et al., 2007) concluded that it is mandatory that normal values for wave reflection indices and central blood pressure must be determined before central blood pressure measurement will be implemented into clinical practice. However, McEniery et al. (2005) presented some normal values for cPP in different age categories.

3.2. Methods

The FLSS was set up for pPP, cPP, PWV and cAI based on data of the ACCT database. The used setup structure is shown in Figure 46.

Data pools: The score calculation is defined by external data (pool of reference values and pool of regression equations) as described in Chapter 2. The pool of reference values serves reference values for male and female subjects. Additionally, unisex reference values based on male and female subjects are presented. The pool of regression lines applies also to male, female and unisex data.

Age groups: Four age groups were defined. Two age groups for middle-aged subjects (g1: 40-49 years, g2: 50-59 years) and two age groups for elderly subjects (g3: 60-69 years, g4: 70-79 years) were defined.

Confounder-correction: The confounder-correction can be optional enabled or disabled. PWV can be corrected for the impact of MAP and cAI can be corrected for the impact of HR and body height.

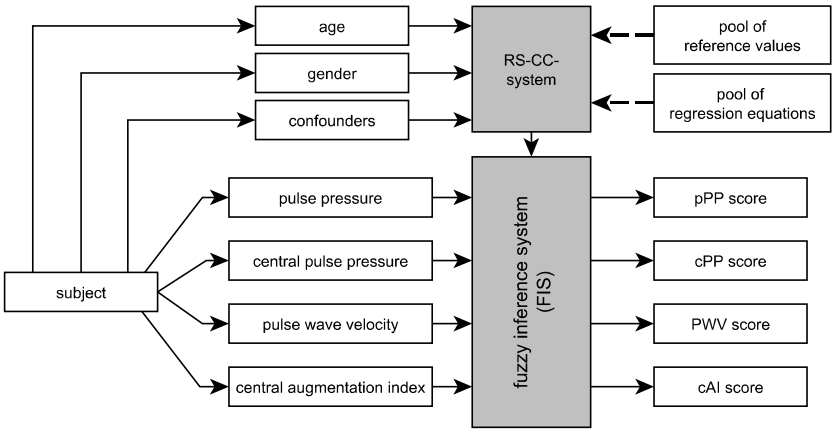


Figure 46: FLSS setup structure. The FLSS was set up for four input variables (pPP, cPP, PWV and cAI).

Statistical analysis: was performed with Statistics Toolbox™ (The MathWorks™) based on MATLAB© software (R2008a, The MathWorks™), PASW Statistics 18 (version 18.0.0, SPSS Inc.) and MedCalc (version 11.5.1.0, MedCalc Software).

3.2.1. Calculation of external data

External data (pool of reference values and pool of regression equations) were calculated based on data from the ACCT database. Therefore, a reference group was extracted by using filter criteria. Only subjects who had normal or borderline normal values for each filter criterion were added to the reference group. The reference group was subsequently used to calculate reference values. Figure 47 summarizes this process.

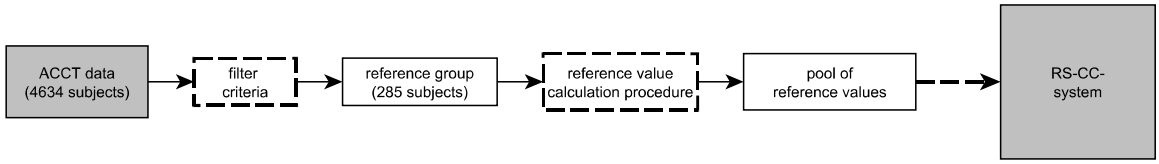


Figure 47: Pool of reference values calculation flow chart. A reference group was extracted from the ACCT data. Reference values were calculated based on the reference group.

Regression lines were calculated based on the extracted reference group for PWV and cAI. Additionally, regression lines were also calculated based on an extended reference group with included hypertensive subjects and on the whole ACCT raw data. Results were compared and interpreted.

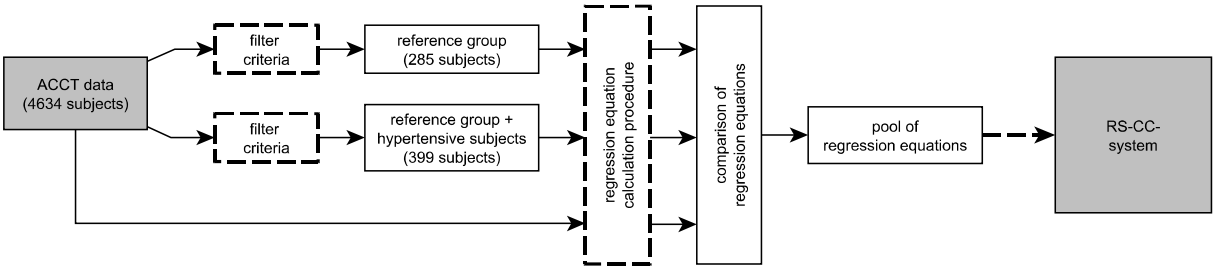


Figure 48: Pool of regression equations calculation flow chart. A reference group was extracted from the ACCT data. Reference values were calculated based on the reference group.

In conclusion, the setup of the FLSS has to be done in the following steps:

1. Extraction of a reference group.
2. Calculation of reference values based on the reference group.
3. Calculation of regression equations to correct for the effect of confounders.
4. Estimation of weighting factors for overall-scores (see Chapter 5).

3.2.1.1. ACCT data

Data were extracted from the ACCT database. The used datasets were different to those used in McEniery et al. (2005). In total 4634 subjects (56% male, 44% female, aged 40 to 92 years) were extracted. Every dataset contained information on: *age, gender, ethnic group, body height, weight, diabetes mellitus, smoking, cardiovascular drugs, TC, LDL cholesterol, HDL cholesterol, triglyceride (TG), glucose, PWV, peripheral SBP, peripheral DBP, MAP, HR, central SBP, central DBP, cAI and central augmentation pressure.* There was also incomplete data on: *stroke volume, cardiac output and cardiac index.* Data values were measured in the supine position. The extracted datasets contain no CVD subjects. Every dataset was reviewed for implausible values (typing errors or implausible high or low values) and datasets with implausible values were excluded.

Central aortic pressure data: Values were determined by arterial tonometry of the radial pulse and a generalized transfer function (Karamanoglu et al., 1993) (see Section 3.1.1.3.1) was used. This

measurement was performed with the SphygmoCor device (AtCor Medical) and data were analyzed with the SphygmoCor software (AtCor Medical).

PWV data: PWV was measured using the foot-to-foot method, gated by ECG as utilized by the SphygmoCor software. The PWV data are carotid-femoral.

3.2.1.2. Reference group extraction procedure

A reference group was extracted by using filter criteria (see Figure 47). Only subjects who had normal or borderline normal values for each filter criterion were added to the reference group. Subjects were filtered based on anthropometric data, blood pressure, blood indicators, medication status and life style factors. Following filter criteria were used:

Filter criteria: *subjects with TC ≥ 6.2 mmol/l, LDL cholesterol ≥ 4.1 mmol/l, HDL cholesterol < 1.0 mmol/l, TG ≥ 2.26 mmol/l, BMI ≥ 30 kg/m^2, peripheral SBP ≥ 140 mmHg, peripheral DBP ≥ 90 mmHg and diabetes mellitus were excluded. Current smokers, past smokers and subjects receiving any medication were also excluded.*

The extracted reference group was subsequently divided into four age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Furthermore, pPP, cPP and PWV were logarithmic normally distributed. For mean and standard deviation the logarithm (natural logarithm) of each dataset of pPP, cPP and PWV was taken and the logarithmic mean μ_{ln} and the logarithmic standard deviation σ_{ln} were calculated. Mean and standard deviation were back transformed with the following equations:

$$\mu^* = e^{\mu_{ln}} \quad \textbf{(44)}$$

$$\sigma^* = \sqrt{\left(e^{2\mu_{ln}+\sigma_{ln}^2}\right)\left(e^{\sigma_{ln}^2}-1\right)} \quad \textbf{(45)}$$

μ_{ln} … logarithmic mean
σ_{ln} … logarithmic standard deviation
μ^* … back-transformed mean
σ^*… back-transformed standard deviation

The same procedure was used to extract an extended reference group with included hypertensive subjects. Therefore no hypertensive subjects were excluded.

3.2.1.3. Reference value calculation procedure

The FLSS uses a pool of reference values for the score calculation as described in Chapter 2. The pool of reference values consists of separated reference values for each variable, decade of age and gender. However, the pool of reference values for pPP, cPP, PWV and cAI were calculated based on the

extracted reference group. A reference value ‘normal’ and a reference value ‘high’ were calculated for each variable, age group and gender.

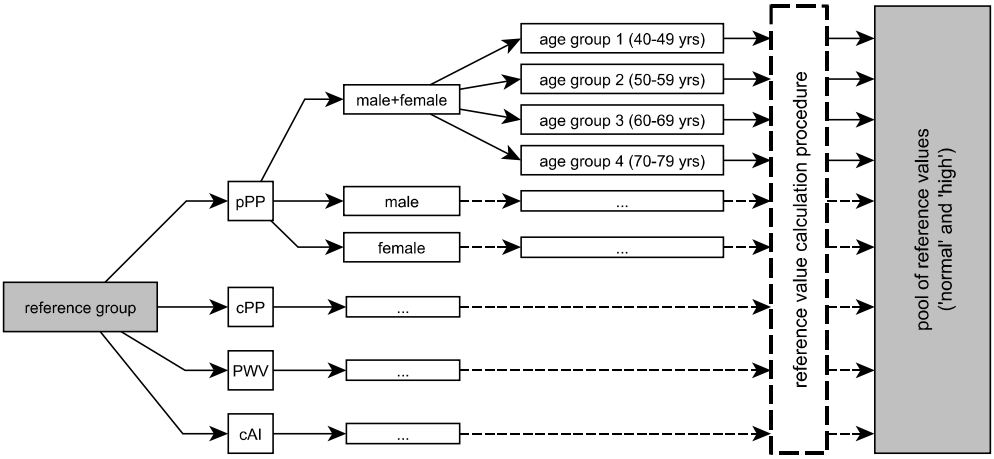


Figure 49: Flow chart of the reference value calculation procedure. The reference group was separated for each variable, gender and age group. For each subgroup a reference value ‘normal’ and a reference value ‘high’ was calculated.

Unfortunately, there was no standardized procedure found in the literature to define a pair of reference values (‘normal’ and ‘high’) based on data of a reference group. Therefore, four different data-based calculation rules were defined and investigated. The aim was to estimate a suitable and generalized calculation rule for the definition of a reference value ‘normal’ and a reference value ‘high’. Each calculation rule was based on statistical parameters such as mean (μ), standard deviation (σ), median ($\tilde{\mu}$), interquartile range IQR ($Q_{0.75}$ - $Q_{0.25}$) or percentile values (P). Rule 1 and 2 were based on mean and standard deviation of a standard normal distribution. An ideal standard normal distribution was assumed. Rule 3 and 4 were based on median, interquartile range and percentile values.

Reference value calculation rule 1: The reference value ‘normal’ and the reference value ‘high’ was defined by following equations:

$$r_{normal} = (\mu + \sigma) * 0.95 \quad \textbf{(46)}$$

$$r_{high} = (\mu + 2\sigma) * 0.95 \quad \textbf{(47)}$$

Reference value calculation rule 2: For calculation rule 2 it was assumed that 55% of a reference group has ‘normal’ and 5% of a reference group has ‘high’ values. Therefore, the reference value ‘normal’ was defined as the distribution value including 55% of the population and the reference value ‘high’ was defined as the distribution value including 95% of the population. For this purpose the following equation was used (area under the standard normal distribution):

$$\Phi(z) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{z} e^{-\frac{1}{2}x^2}\, dx \quad \textbf{(48)}$$

A z-value of 0.13 leads to a population under the curve of 55% ($\Phi(0.13) \approx 0.55$) and a z-value of 1.65 leads to a population under the curve of 95% ($\Phi(1.65) \approx 0.95$):

In conclusion the reference values ‘normal’ and ‘high’ can be calculated with following equations:

$$r_{normal} = \mu + 0.13\,\sigma \quad \textbf{(49)}$$

$$r_{high} = \mu + 1.65\,\sigma \quad \textbf{(50)}$$

Reference value calculation rule 3: The reference value ‘normal’ was defined as the percentile value P75 which equals the third quartile value Q3 ($Q_{0.75}$). The reference value ‘high’ was calculated by following equation:

$$r_{high} = \tilde{\mu} + 1.5 * (Q_{0.75} - Q_{0.25}) \quad \textbf{(51)}$$

Reference value calculation rule 4: The reference value ‘normal’ was defined as the percentile value P55 and the reference value ‘high’ was the percentile value P95. This definition was chosen similar to reference value calculation rule 2.

3.2.1.4. Regression equation calculation procedure

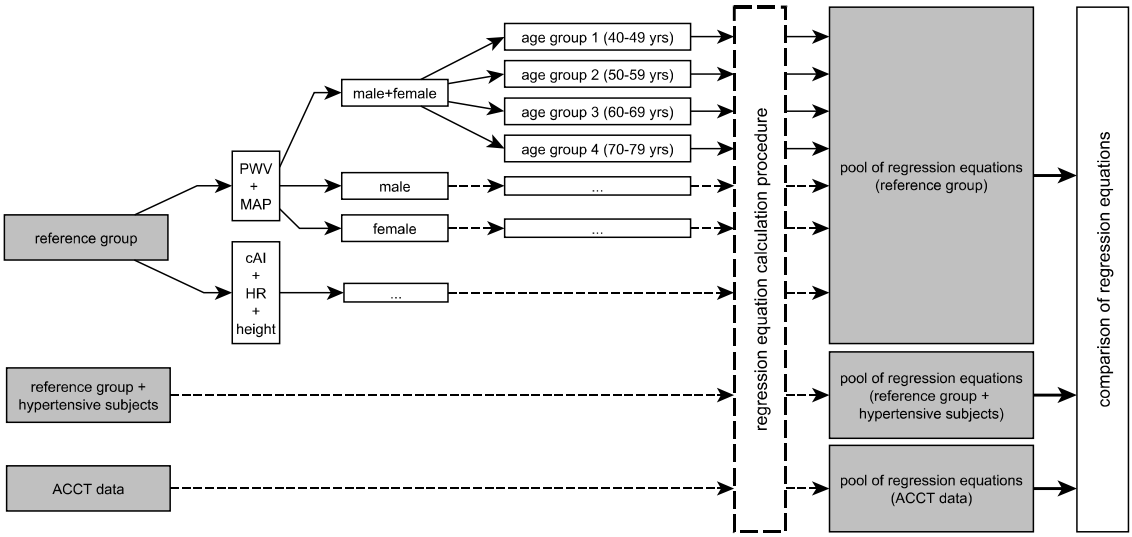


Figure 50: Flow chart of the calculation of regression equations. Each group was separated for each variable (PWV and cAI), confounder, gender and age group. For each subgroup a regression equation which describes the dependency between variable and confounder was calculated. This procedure was used for three different groups of data.

No intra-individual data were available to calculate the dependencies between variables and confounders for different age groups and gender. Hence, the relationship between variables and confounders were estimated by inter-individual data (ACCT data). However, the FLSS uses multiple linear regression equations for the confounder-correction as shown in Section 2.2.2.3.1. The pool of regression equations consists of separated regression equations for each variable (PWV and cAI), decade of age and gender. Regression equations were calculated for the relationship between PWV and MAP, and further for the relationship between cAI, HR and body height. Furthermore, three

different groups (reference group, reference group with included hypertensive subjects and the whole ACCT data) were used. This was done to analyze if there is a difference in the inter-individual confounder-dependencies between subgroups. Figure 50 summarizes this procedure.

The pool of regression equations consists of a set of multiple linear regression equations which are used to shift the selected pair of reference values to lower or higher values to correct for the effect of confounders (see Section 2.2.2.3). Therefore, a shift (Δr) is calculated by a multiple linear regression equation as shown in (31)-(33). In this investigation the following equations were defined to calculate the reference value shift:

$$\Delta r_{PWV} = (a_0 + a_{MAP} \cdot MAP) - (a_0 + a_{MAP} \cdot b_{MAP}) \quad \textbf{(52)}$$

$$\Delta r_{AI} = \left(a_0 + a_{HR} \cdot HR + a_{height} \cdot height\right) - \left(a_0 + a_{HR} \cdot b_{HR} + a_{height} \cdot b_{height}\right) \quad \textbf{(53)}$$

The regression coefficients are: a_{MAP}, a_{HR}, a_{height}. The reference values for the confounders (MAP, HR, height) are: b_{MAP}, b_{HR}, b_{height}. These reference values were calculated as the mean of each variable of the whole ACCT data. Following reference values were defined: b_{MAP} = 95 mmHg, b_{HR} = 65 bpm, b_{height} = 1.7 m.

3.2.1.5. Confounder correction of reference values

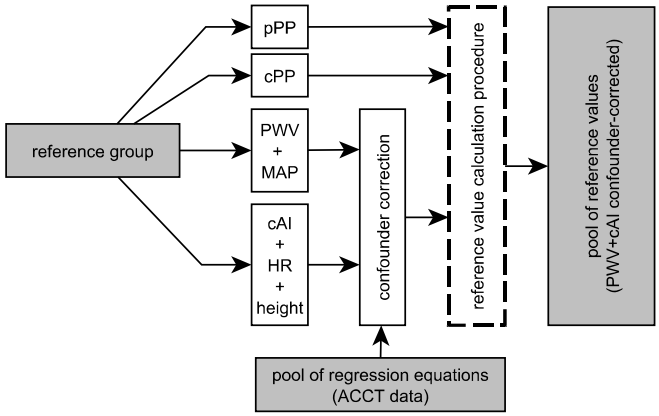


Figure 51: Flow chart of the calculation of confounder-corrected reference values for PWV and cAI. Data were separated for each variable. PWV data were corrected for MAP and cAI data were corrected for HR and body height.

Reference values for PWV and cAI were calculated based on the extracted reference group. In addition, confounder-corrected reference values for cAI and PWV were calculated and investigated. Therefore, the data of PWV and cAI of the reference group were corrected for the impact of confounders. Therefore, the pool of regression equations calculated by the ACCT data was used to correct the data of PWV and cAI for the impact of their confounders (MAP, HR and body height).

Subsequently, reference values were calculated based on this confounder-corrected data. Data of pPP and cPP were unchanged.

Confounder correction of data: The confounder correction of data of PWV and cAI was calculated with equation (54). This equation calculates the difference (Δy_k) between each dataset of the unadjusted dependent variable (y_k) and the value calculated by the regression equation. The value of the regression equations is calculated by the associated values of the confounders (c_{jk}) of each dataset.

$$\Delta y_k = y_k - \left(a_0 + a_1 c_{1k} + a_2 c_{2k} + \cdots + a_j c_{jk} + \cdots a_J c_{Jk}\right) \quad \textbf{(54)}$$

y_k = value of uncorrected dependent variable (k = 1, 2, …, K)
Δy_k = difference of uncorrected dependent variable and estimated dependent variable (k = 1, 2,…, K)

The following equation calculates the confounder-corrected dataset. Therefore, a baseline value based on the reference values of the confounders (b_j) is calculated by the regression equation. The calculated difference Δy_k is summated to the baseline value.

$$y_k^* = \left(a_0 + a_1 b_1 + a_2 b_2 + \cdots + a_j b_j + \cdots a_J b_J\right) + \Delta y_k \quad \textbf{(55)}$$

y_k^* = values of corrected variable (corrected dataset) (k = 1, 2, …, K)

3.3. Results

3.3.1. Group data

3.3.1.1. ACCT data

The ACCT data consists of 4634 subjects aged from 40 to 92 years. Only data of subjects aged 40 to 79 years (4368 subjects) were used for the setup and investigation of the FLSS. In summary, 8.4 % of the investigated subjects were aged 40 to 49 years, 16.7 % were aged 50 to 59 years, 41.0 % were aged 60 to 69 years and 33.9 % were aged 70 to 79 years. Hence, the used ACCT data consist predominantly of older subjects. Data were separated into four different age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and three gender categories (male + female subjects, male subjects, female subjects) were defined. A detailed summary statistic for each variable and subgroup can be found in the appendix (see Table 8). Box-whisker-plots of pPP, cPP, PWV and cAI are shown in following figures:

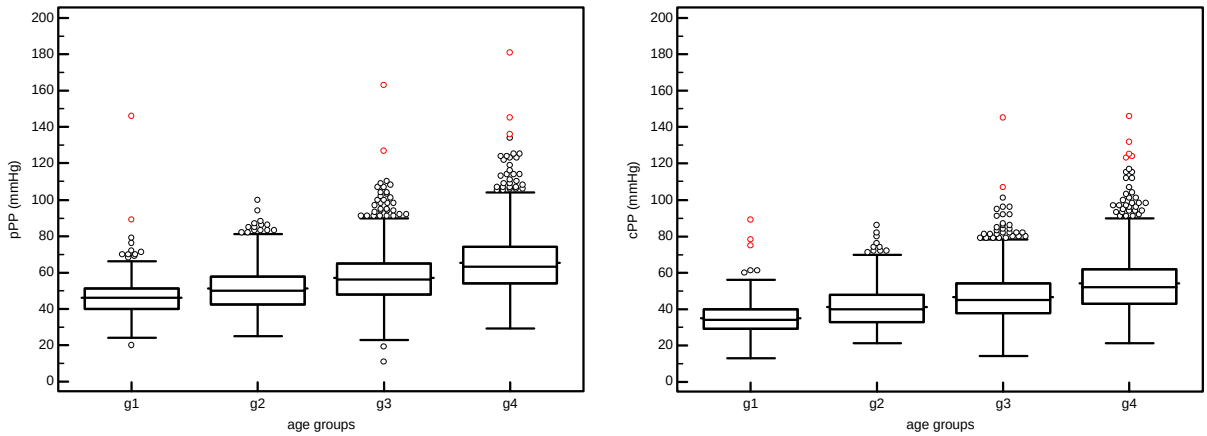


Figure 52: Box-whisker-plot of pPP and cPP (ACCT data). The left figure shows age group characteristics for pPP and the right figure shows age group characteristics for cPP. Results are separated for: g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Group characteristics were calculated by unisex data (male + female subjects).

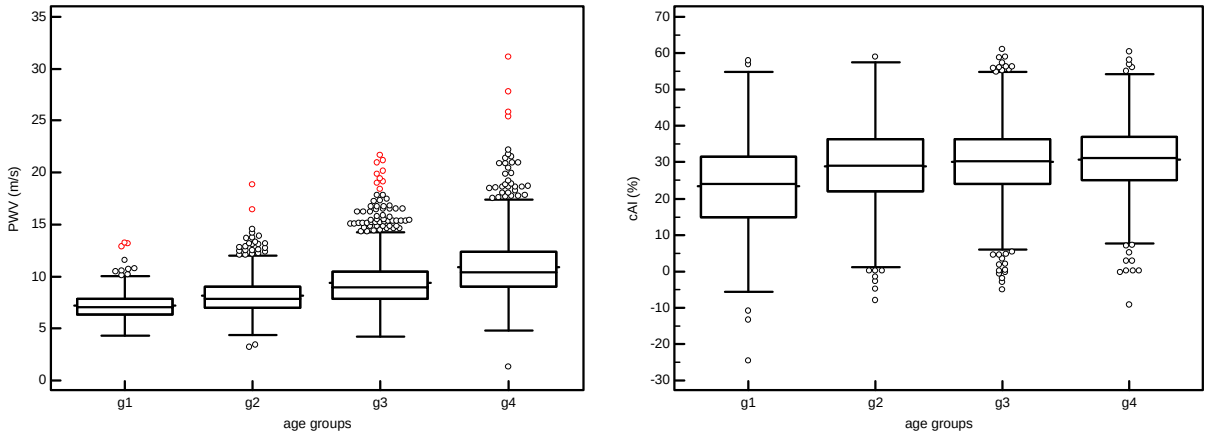


Figure 53: Box-whisker-plot of PWV and cAI (ACCT data). The left figure shows age group characteristics for PWV and the right figure shows age group characteristics for cAI. Results are separated for: g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Group characteristics were calculated by unisex data (male + female subjects).

For pPP, cPP and PWV, mean and standard deviation of the ACCT data increased progressively with age. The variable pPP was higher than cPP in all age groups. The variable cAI increased from age group 1 (40-49 years) to age group 3 (60-69 years) but cAI was nearly unchanged between age group 3 (60-69 years) and age group 4 (70-79 years). Hence, cAI showed a plateau effect for older subjects. The standard deviation of cAI decreased with age. Same results were found for the median and interquartile range.

3.3.1.2. Reference group data

In total 285 subjects aged 40 to 83 years of the ACCT data (4634 subjects) satisfied the filter criteria. Only data of subjects aged 40 to 79 years (277 subjects) were used. A detailed summary statistic for each variable and subgroup can be found in the appendix (see Table 9-Table 12). The variables pPP, cPP and PWV were normally-distributed after logarithmic-transformation. Box-whisker-plots of pPP, cPP, PWV and cAI are shown in following figures:

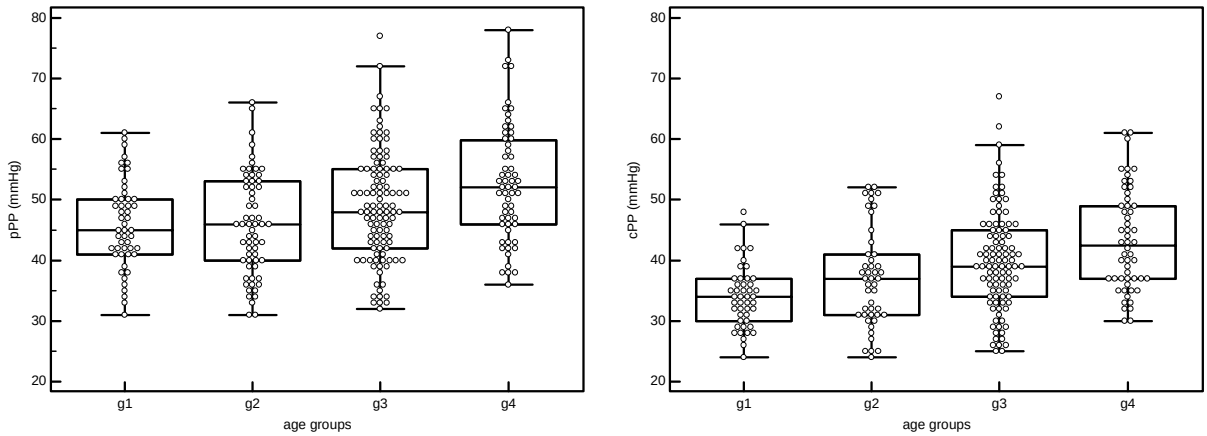


Figure 54: Box-whisker-plot of pPP and cPP (reference group). The left figure shows age group characteristics for pPP and the right figure shows age group characteristics for cPP. Results are separated for: g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Group characteristics were calculated by unisex data (male + female subjects).

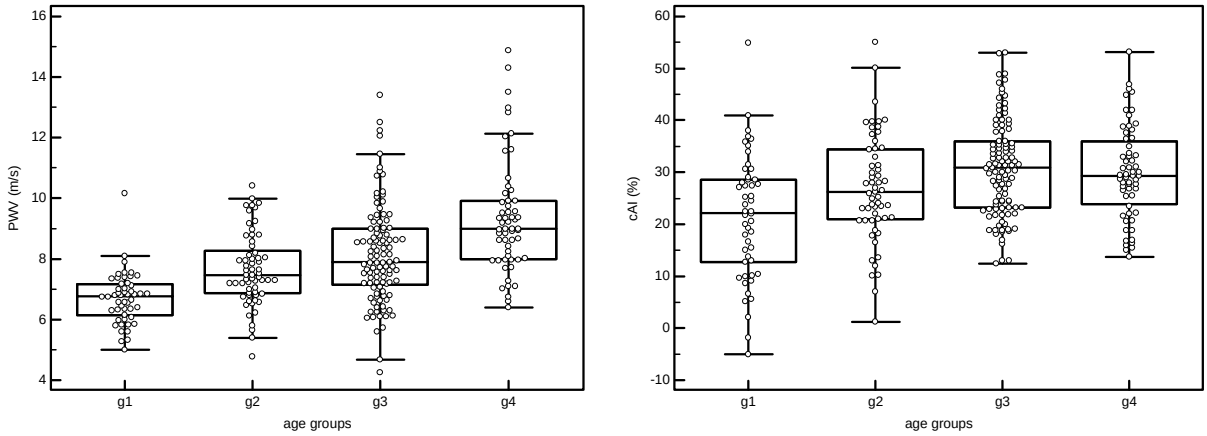


Figure 55: Box-whisker-plot of PWV and cAI (reference group). The left figure shows age group characteristics for PWV and the right figure shows age group characteristics for cAI. Results are separated for: g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Group characteristics were calculated by unisex data (male + female subjects). Uncorrected PWV and cAI data were used.

The variable pPP increased predominantly over 60 years of age, whereas cPP increased progressively with age. Hence, pPP showed a plateau effect for younger subjects. PWV increased progressively with age as well. However, cAI showed the same plateau effect for elderly subjects as found in the ACCT data.

Reference group versus ACCT data: Mean and median of pPP were lower for all subgroups (age groups and gender groups) in the reference group, especially for subjects older than 50 years. In addition, mean and median of pPP for subjects aged 40 to 49 years calculated by the reference group were similar to that calculated by the ACCT data. Similar results were found for cPP, although differences were higher for younger subjects as shown for pPP. Mean and median of PWV were lower for all subgroups in the reference group. The mean and median of cAI were lower for younger subjects (g1: 40-49 years, g2: 50-59 years) in the reference group whereas the mean and median was nearly unchanged between the reference group and the ACCT data for older subjects (g3: 50-59 years, g4: 60-69 years).

3.3.1.2.1. Gender-specific results

Gender specific group characteristics of the reference group can be found in the appendix (see Table 9-Table 12). Gender-specific box-whisker-plots of pPP, cPP, PWV and cAI are shown in following figures:

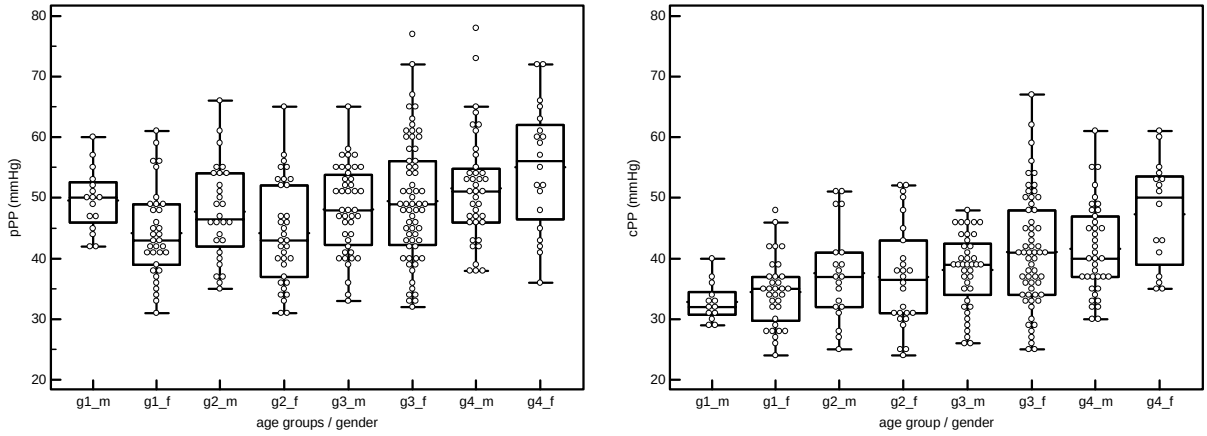


Figure 56: Gender-specific box-whisker-plot of pPP and cPP (reference group). The left figure shows age group and gender group characteristics for pPP. The right figure shows age group and gender group characteristics for cPP. Results are separated for age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender (m: male; f: female).

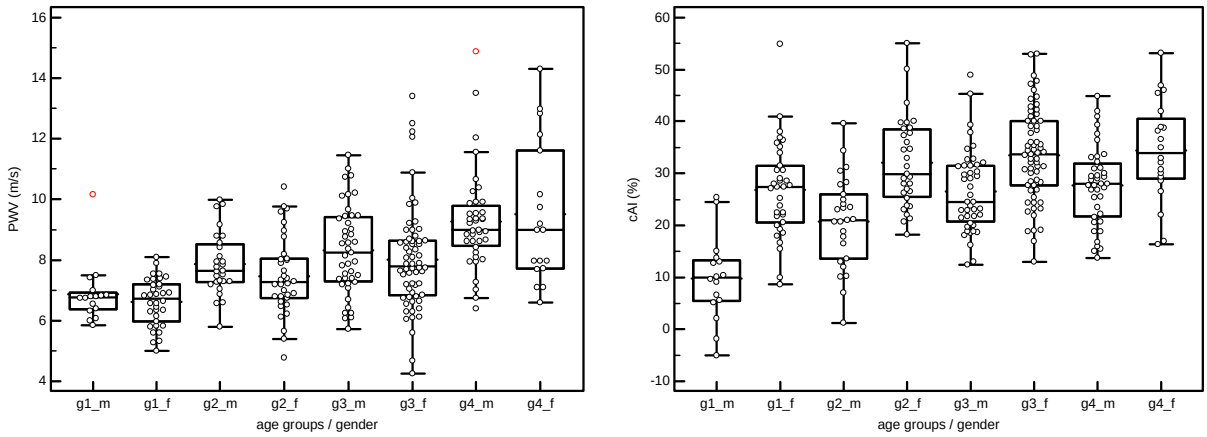


Figure 57: Gender-specific box-whisker-plot of PWV and cAI (reference group). The left figure shows age group and gender group characteristics for PWV. The right figure shows age group and gender group characteristics for cAI. Results are separated for age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender (m: male; f: female).

Mean and median of pPP were higher for male subjects in age group 1 and age group 2, whereas mean and median were higher for female subjects in age group 3 and age group 4. Further pPP did not increase between age group 1 and age group 2 (plateau effect) for female subjects whereas male subjects showed a decrease between age group 1 and age group 2. In contrast, mean and median for cPP increased progressively with age. Mean and median were higher for female subjects in age group 1, age group 3 and age group 4. Interestingly, mean and median of cPP were higher for male subjects in age group 2. The mean and median for PWV were higher for male subjects in all age groups. Mean and median for cAI were higher for females in all age groups. Interestingly, mean and median of cAI was markedly lower for male subjects in age group 1.

3.3.1.2.2. Confounder-corrected data

PWV and cAI data of the reference group were confounder-corrected (PWV data were corrected for MAP and cAI data were corrected for HR and body height). A detailed summary statistic can be found in the appendix (see Table 13 and Table 14). Following figures show box-whisker-plots of the confounder-corrected reference group data for PWV and cAI:

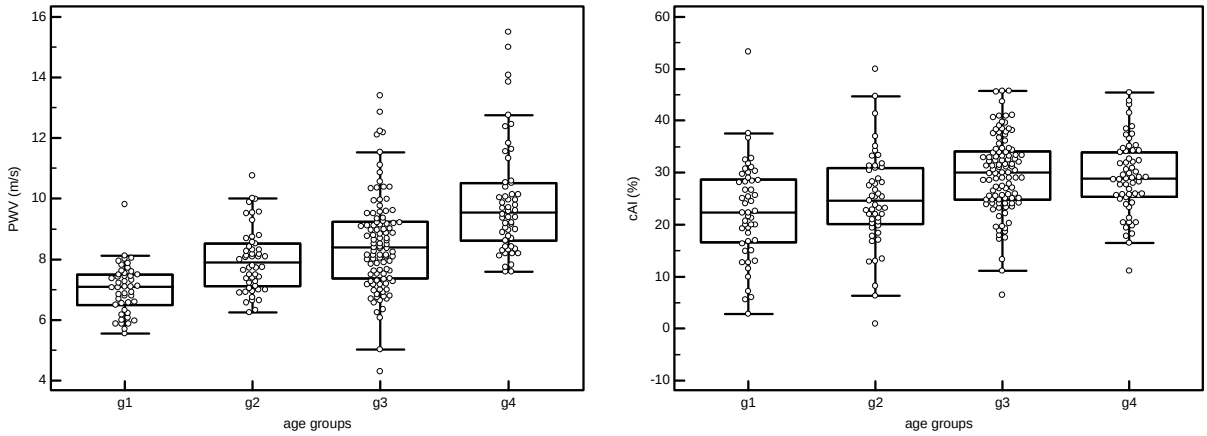


Figure 58: Box-whisker-plot of PWV and cAI (reference group – confounder corrected). The left figure shows age group characteristics for PWV. The right figure shows age group characteristics for cAI. Results are separated for age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Group characteristics were calculated by unisex data (male + female subjects). Confounder-corrected data was used (PWV was corrected for MAP, HR was corrected for HR and body height).

Confounder-corrected reference group versus uncorrected reference group: Mean and median of PWV were higher for all subgroups (age groups and gender groups) in the confounder-corrected reference group. Mean and median of cAI were lower for female subjects in the confounder-corrected reference group. Mean and median of cAI showed a tendency to remain unchanged or be higher for male subjects. Hence, the difference between male and female subjects was lower in the confounder-corrected reference group compared to the uncorrected reference group.

3.3.1.3. Reference group with included hypertensive subjects

Regression lines for PWV were calculated based on a reference group with included hypertensive subjects. A detailed summary statistic can be found in the appendix (see Table 15). Figure 59 shows the box-whisker-plot of the reference group with included hypertensive subjects for PWV.

Reference group versus reference group with included hypertensive subjects: Mean and median of PWV were higher for all subgroups (age groups and gender groups) in the reference group with included hypertensive subjects, especially for subjects older than 60 years.

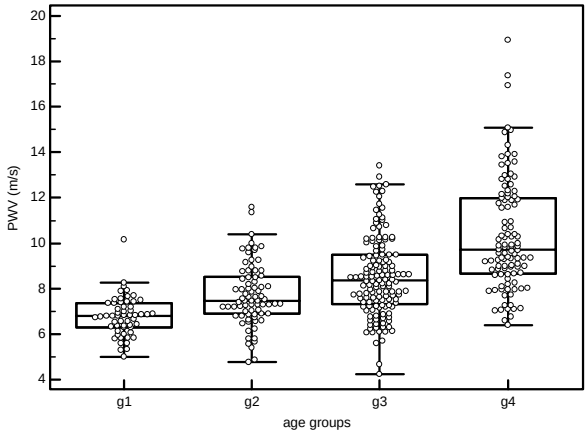


Figure 59: Box-whisker-plot of PWV (reference group with included hypertensive subjects). Results are separated for age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Group characteristics were calculated by unisex data (male + female subjects).

3.3.2. Reference values

A pair of reference values (‘normal’ and ‘high’) was calculated for each variable, decade of age and gender. Four different reference value calculation rules were investigated. Calculated reference values and can be found in the appendix (see Table 19-Table 22). The following figures show an overview about the resulted reference values. Each subplot presents the reference values for a specific variable and calculation rule. Each bar presents the pair of reference values for a specific age group. The top of the white bars indicate the reference value ‘normal’ and the top of the grey bars indicate the reference value ‘high’. White areas can be interpreted as ‘normal’ values, grey areas as ‘elevated’ values and black areas as ‘high’ values.

Figure 60 shows the calculated unisex reference values for pPP, cPP, PWV and cAI based on the reference group. For each calculation rule, the reference values for pPP, cPP and PWV increased with age and the interval of elevated values showed a widening with age. In contrast, the reference values for cAI showed an increase for younger subjects and a slight decrease for older subjects.

Comparison of calculation rules for pPP, cPP, PWV and cAI: Rule 1 and Rule 3 resulted in higher reference values for ‘normal’ than rule 2 and 4. In contrast, rule 1 and rule 2 resulted lower reference values for ‘high’ than rule 3. Additionally, rule 4 resulted higher reference values for ‘high’ than rule 2 and 4, especially for older subjects.

In conclusion:
Rule 1 leads to high reference values for ‘normal’ and low reference values for ‘high’.
Rule 2 leads to low reference values for ‘normal’ and low reference values for ‘high’.
Rule 3 leads to high reference values for ‘normal’ and high reference values for ‘high’.
Rule 4 leads to low reference values for ‘normal’ and high reference values for ‘high’, especially for older subjects.

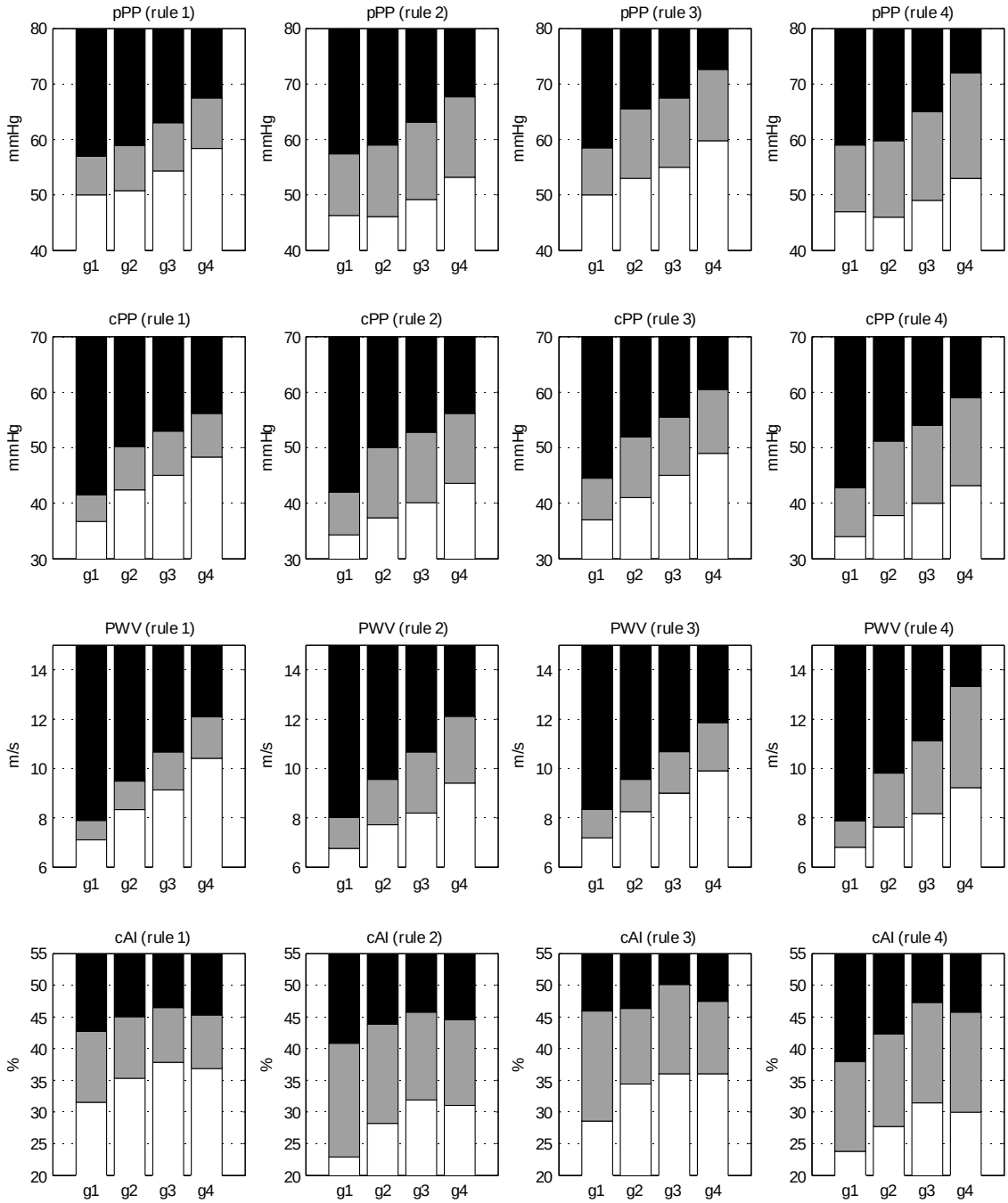


Figure 60: Plot of calculated reference values for pPP, cPP, PWV and cAI. Presented reference values were calculated based on the reference group. Unisex data (male + female subjects) were used. Calculated reference values are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and used calculation rule (1-4). The top of the white bars indicate the reference value 'normal' and the top of the grey bars indicate the reference value 'high'. White areas can be interpreted as 'normal' values, grey areas as 'elevated' values and black areas as 'high' values.

For pPP, calculation rule 1 and 2 showed a plateau effect for younger subjects. That means, reference values for 'normal' are similar for age group 1 and age group 2. For cAI, a plateau effect for older subjects was shown for each calculation rule. Interestingly, reference values for 'high' calculated by calculation rule 3 showed similar reference values for each age group except for age group 3.

3.3.2.1. Gender-specific results

Figure 61 shows gender-specific reference values for pPP, cPP, PWV and cAI calculated by reference value calculation rule 1 and 2. Figure 62 shows gender-specific reference values for pPP, cPP, PWV and cAI calculated by reference value calculation rule 3 and 4.

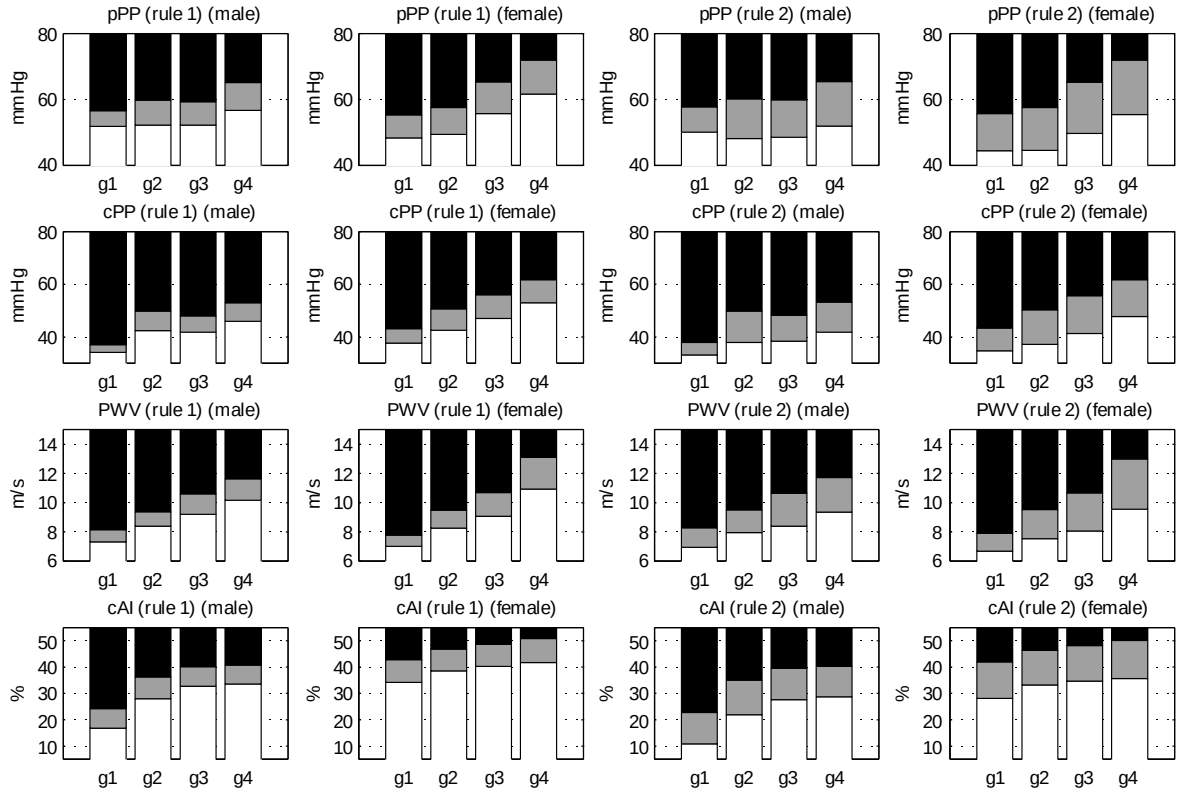


Figure 61: Plot of calculated gender-specific reference values for pPP, cPP, PWV and cAI (rule 1 and rule 2). Presented reference values were calculated based on the reference group. Reference value calculation rule 1 and 2 were used and reference values were calculated separately for male and female subjects. Calculated reference values are presented separately for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). The top of the white bars indicate the reference value 'normal' and the top of the grey bars indicate the reference value 'high'. White areas can be interpreted as 'normal' values, grey areas as 'elevated' values and black areas as 'high' values.

It is important to note that gender-specific results have uncertainties due to the low number of subjects per subgroup. In fact, gender-specific reference values for pPP, cPP and PWV were in some cases lower for higher age groups. For example, the reference value 'high' for cPP was lower in age group 3 than in age group 2 calculated by calculation rule 4.

Comparison of calculation rules for pPP, cPP, PWV and cAI: Gender-specific reference values calculated by calculation rule 3 and 4 showed strong uncertainties. Gender-specific reference values calculated by calculation rule 1 and 2 showed better results.

Comparison of male and female results: In conclusion, reference values for pPP were higher for young male (age group 1, age group 2) and old female subjects (age group 3, age group 4). Reference values for cPP were higher for female subjects. Reference values for PWV were in some groups higher for

male and in some groups higher for female subjects depending on the used calculation rule. Reference values for cAI were higher for female subjects.

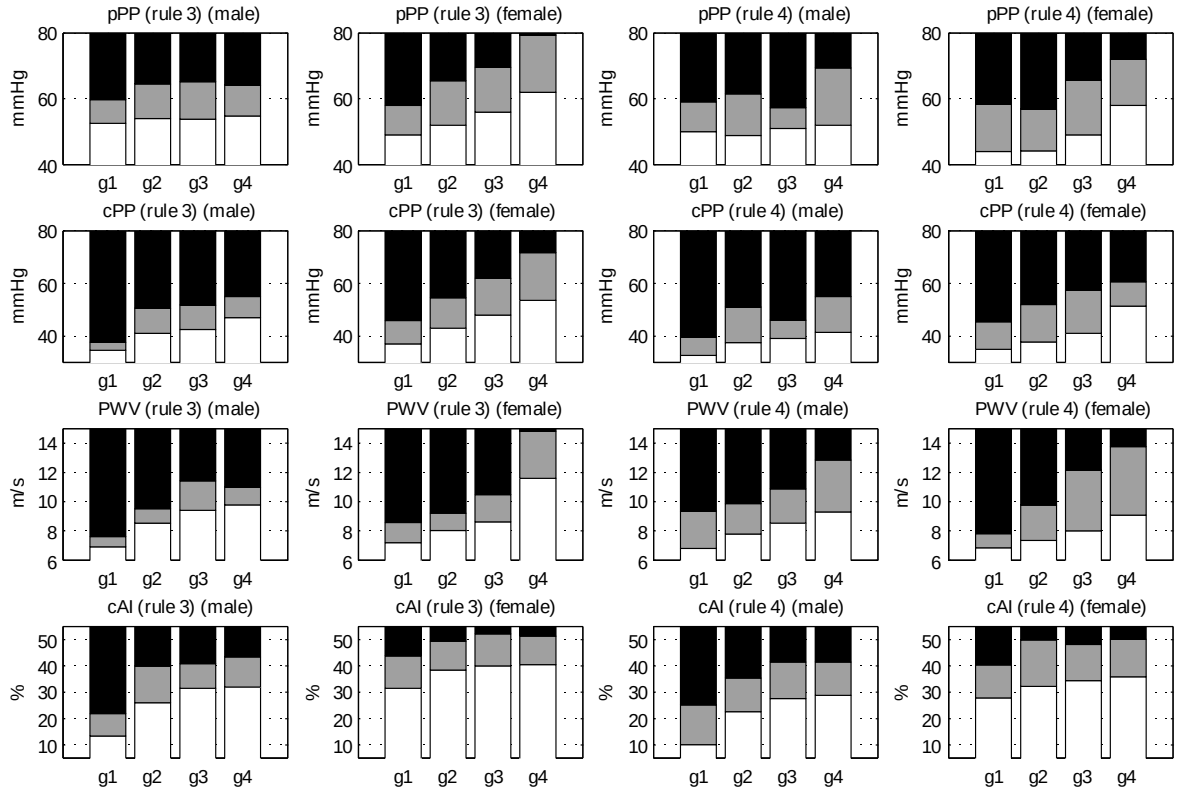


Figure 62: Plot of calculated gender-specific reference values for pPP, cPP, PWV and cAI (rule 3 and rule 4). Presented reference values were calculated based on the reference group. Reference value calculation rule 3 and 4 were used and reference values were calculated separately for male and female subjects. Calculated reference values are presented separately for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). The top of the white bars indicate the reference value 'normal' and the top of the grey bars indicate the reference value 'high'. White areas can be interpreted as 'normal' values, grey areas as 'elevated' values and black areas as 'high' values.

3.3.2.2. Confounder-corrected reference values

Additional reference values for PWV and cAI were calculated based on confounder-corrected data of PWV and cAI of the reference group. Figure 63 shows the calculated unisex reference values for pPP, cPP, PWV and cAI. Calculated reference values can be found in the appendix (Table 23).

Most unisex reference values for PWV were higher in comparison to reference values calculated based on uncorrected reference group data. Also separated reference values for male and female subjects showed a tendency to be higher. In contrast, most unisex reference values for cAI were lower in comparison to reference values calculated on uncorrected reference group data. Further reference values (cAI) for male subjects showed a tendency to be higher or unchanged and reference values (cAI) for female subjects showed a tendency to be lower.

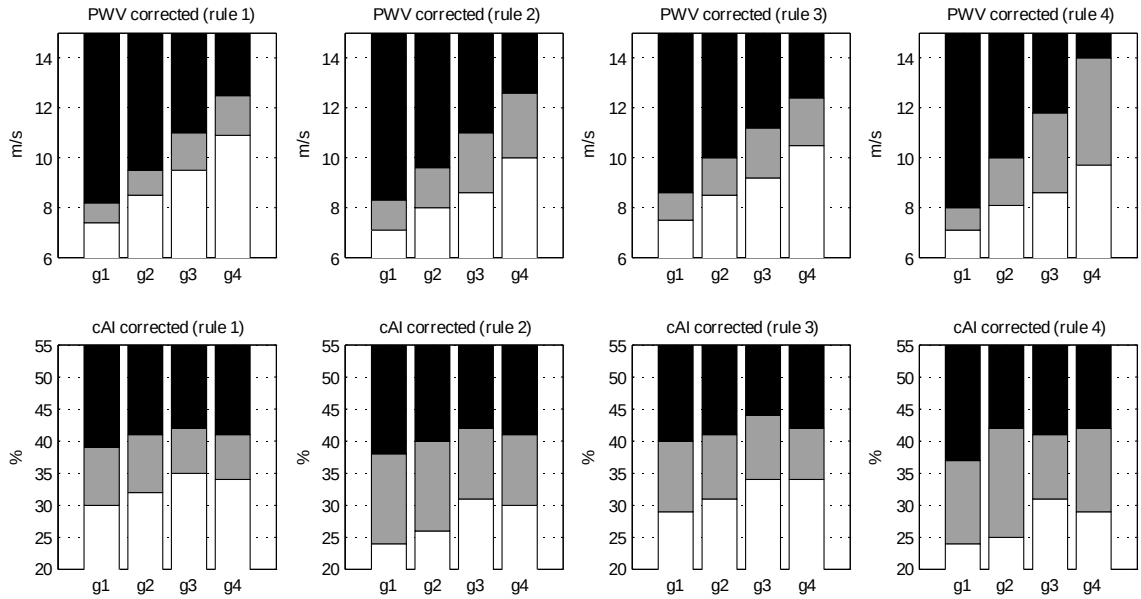


Figure 63: Plot of calculated reference values for PWV and cAI based on confounder-corrected data. Presented reference values were calculated based on the confounder-corrected data of PWV and cAI of the reference group. Unisex data (male + female subjects) was used. Calculated reference values are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and used calculation rule (1-4). The top of the white bars indicate the reference value 'normal' and the top of the grey bars indicate the reference value 'high'. White areas can be interpreted as 'normal' values, grey areas as 'elevated' values and black areas as 'high' values.

3.3.3. Regression equations

Regression equations were calculated to account for the effects of confounders. PWV was investigated for the impact of MAP, and cAI was investigated for the impact of HR and body height. Calculated regression equations and a detailed regression statistic can be found in the appendix (see Table 24 and Table 25). Results are separated for decades of age, gender and the used data for the calculation.

3.3.3.1. PWV depending on MAP

Regression equations for PWV were calculated based on three different groups of data: ACCT data, reference group and reference group with included hypertensive subjects. Calculated regression equations can be found in the appendix (Table 24). The following figure shows the calculated regression equations for PWV based on the ACCT data:

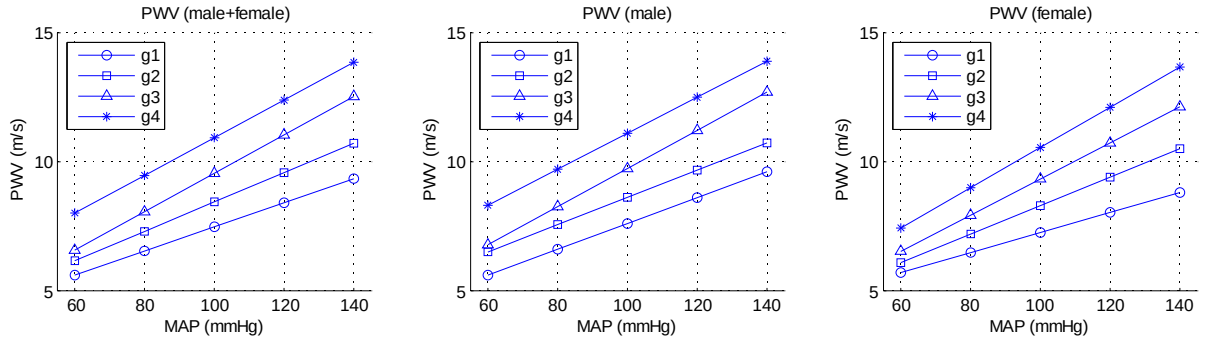


Figure 64: Plot of regression lines for PWV depending on MAP (ACCT data). Calculated regression lines are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender category. Blue lines (–) are calculated regression lines with a p-value < 0.01.

PWV was positively and significantly (p-value < 0.01) dependent on MAP for all age groups and this relationship became more prominent with increasing age. There were only slight differences between male and female subjects. However, the main differences were found for age group 4.

The following figure shows the calculated regression equations for PWV based on the reference group:

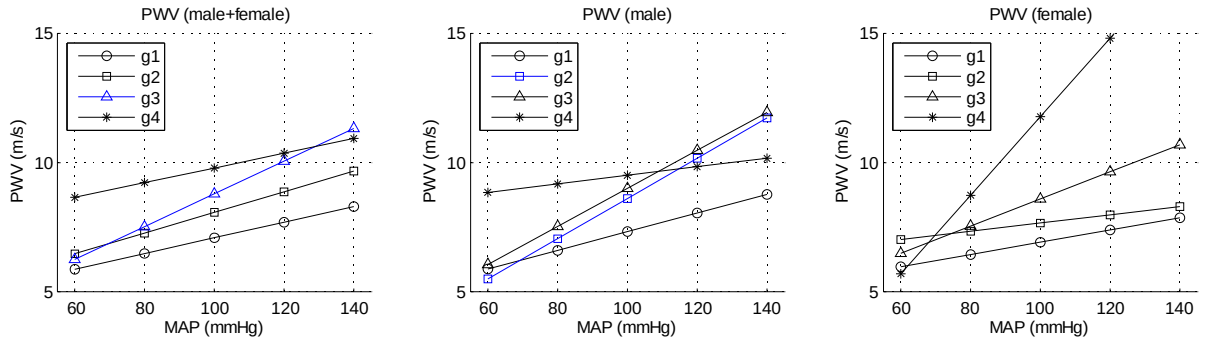


Figure 65: Plot of regression lines for PWV depending on MAP (reference group). Calculated regression lines are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender category. Blue lines (–) are calculated regression lines with a p-value < 0.01.

PWV was positively dependent on MAP for all age groups and gender categories. Most results were not significant (p-value < 0.01). It is important to note that due to the used filter criteria no hypertensive subjects were included to the reference group. Therefore, a reference group with included hypertensive subjects was used for a further investigation.

The following figure shows the calculated regression equations for PWV based on the reference group with included hypertensive subjects:

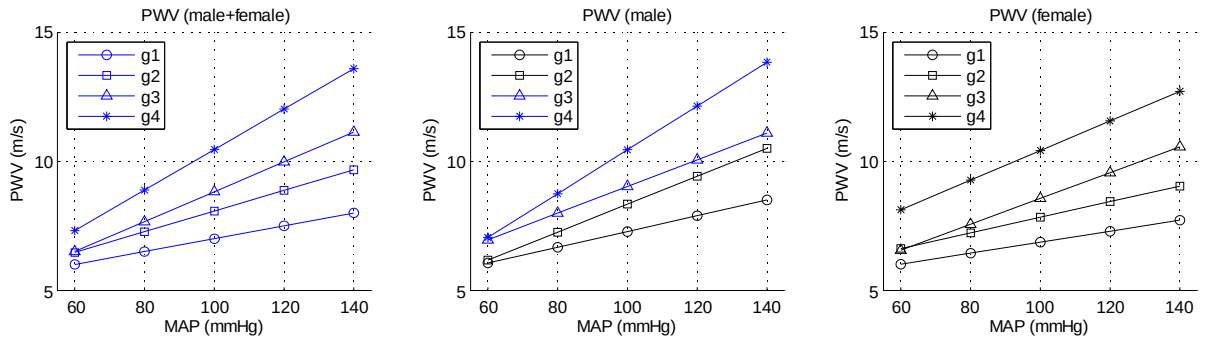


Figure 66: Plot of regression lines for PWV depending on MAP (reference group with included hypertensive subjects). Calculated regression lines are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender category. Blue lines (–) are calculated regression lines with a p-value < 0.01.

This figure shows similar relationships between PWV and mean arterial pressure as shown for the ACCT data. Although the increase of PWV due to an increase of MAP was steeper for some subgroups compared to the ACCT data. This was found especially for unisex data and male data (age group 3 and age group 4). All regression equations calculated based on unisex data were significant (p < 0.01). Regression equations calculated based on male data were only significant for age group 1 and age group 2. Regression equations calculated based on female data were not significant.

3.3.3.2. cAI depending on HR and body height

Regression equations for cAI were calculated based on two different groups of data: ACCT data and reference group. The effect of HR and body height was investigated. Calculated regression equations can be found in the appendix (see Table 25).

ACCT data: For each gender and age category, cAI was negatively and significantly ($p < 0.01$) dependent on HR and body height. Figure 67 shows the calculated dependencies of cAI over HR for a constant height-value of 1.7 m and Figure 68 shows the calculated dependencies of cAI over body height for a constant HR-value of 65 bpm.

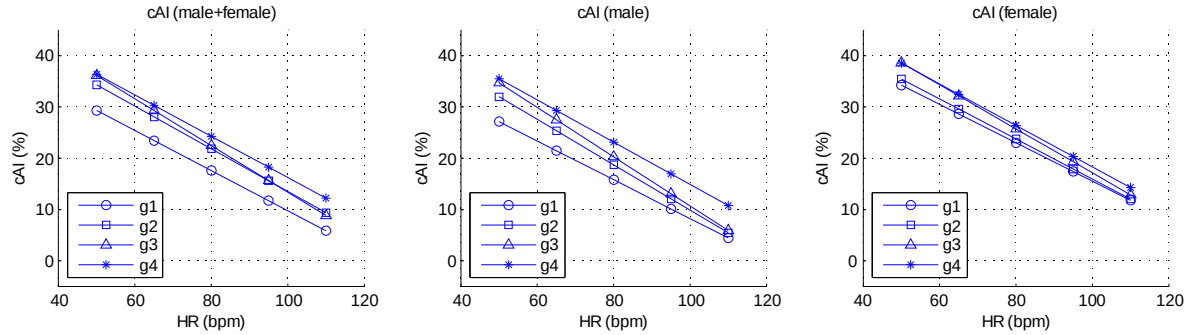


Figure 67: Plot of regression lines for cAI depending on HR (ACCT data). Calculated regression lines are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender category. Blue lines (–) are calculated regression lines with a p-value < 0.01.

cAI and HR: The relationship between cAI and HR was shifted slightly to higher cAI values with increasing age. The gradient of the regression line was nearly unchanged between age groups. The main difference between male and female subjects was that regression lines of female subjects were shifted to higher cAI values compared to male subjects. Furthermore, the regression lines of male subjects were slightly steeper.

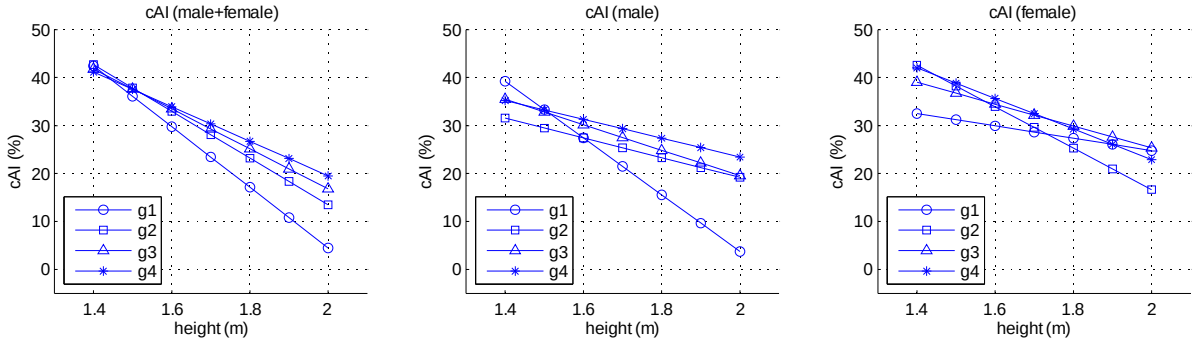


Figure 68: Plot of regression lines for cAI depending on body height (ACCT data). Calculated regression lines are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender category. Blue lines (–) are calculated regression lines with a p-value < 0.01.

cAI and body height: The negative gradient of the regression line increased in magnitude with age for male subjects and unisex data. That is, a change in body height leads to a higher change in cAI for younger subjects. Additionally, male subjects showed a much steeper regression line for age group 1

in comparison to female subjects. For age group 2-3, male and female subjects showed similar regression lines.

Reference group: For unisex data, cAI was negatively and significantly ($p < 0.01$) dependent on HR and body height for each age group. For male and female subjects, only regression equations of age group 3 and age group 4 were significant. Figure 69 shows the calculated dependencies of cAI over HR for a constant height-value of 1.7 m. Figure 70 shows the calculated dependencies of cAI over body height for a constant HR-value of 65 bpm.

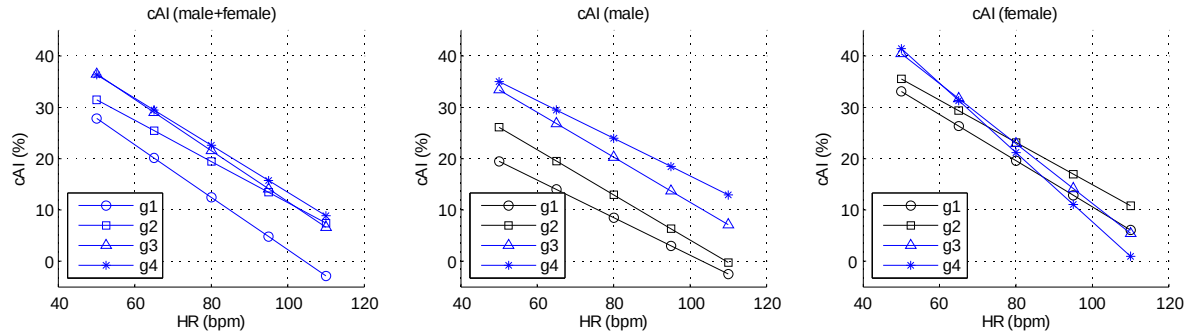


Figure 69: Plot of regression lines for cAI depending on HR (reference group). Calculated regression lines are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender category. Blue lines (–) are calculated regression lines with a p-value < 0.01.

cAI and HR: For unisex data, the relationship between cAI and HR was shifted to higher cAI values with increasing age. The gradient of the regression line was nearly unchanged between age groups. However, regression lines were steeper compared to the regression lines calculated for the ACCT data. Regression lines for male subjects were shifted to lower values for age group 1 and age group 2 compared to the ACCT data. Regression lines of female subjects were much steeper compared to the ACCT data.

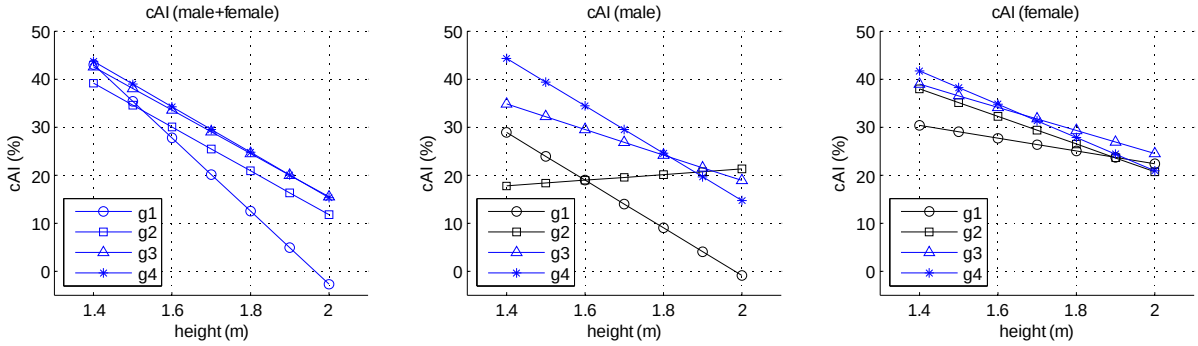


Figure 70: Plot of regression lines for cAI depending on body height (reference group). Calculated regression lines are separated for each age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years) and gender category. Blue lines (–) are calculated lines equations with a p-value < 0.01.

cAI and body height: For unisex data, resulted regression lines were similar compared to regression lines calculated by the ACCT data. Regression lines calculated for male subjects (age group 1 and age group 2) were markedly different compared to the ACCT data but these regression lines were not significant ($p > 0.01$). Regression lines calculated for female subjects showed only slight differences compared to the ACCT data.

3.3.4. Behavior of the FLSS

The used setup structure and the calculated external data leads to a specific score calculation behavior. This section presents examples of the resulted score calculation behavior.

3.3.4.1. Age-dependency

Figure 71 shows the resulting score ramps for the input variables (pPP, cPP, PWV and cAI) categorized by each defined age category (age group 1: 40-49 years, age group 2: 50-59 years, age group 3: 60-69 years, age group 4: 70-79 years). Each score ramp is defined by the associated pair of reference values extracted from the pool of reference values. Unisex reference values were used for this example. Further it is shown that score ramps are smooth sigmoidal functions due to the calculation dynamic of fuzzy logic.

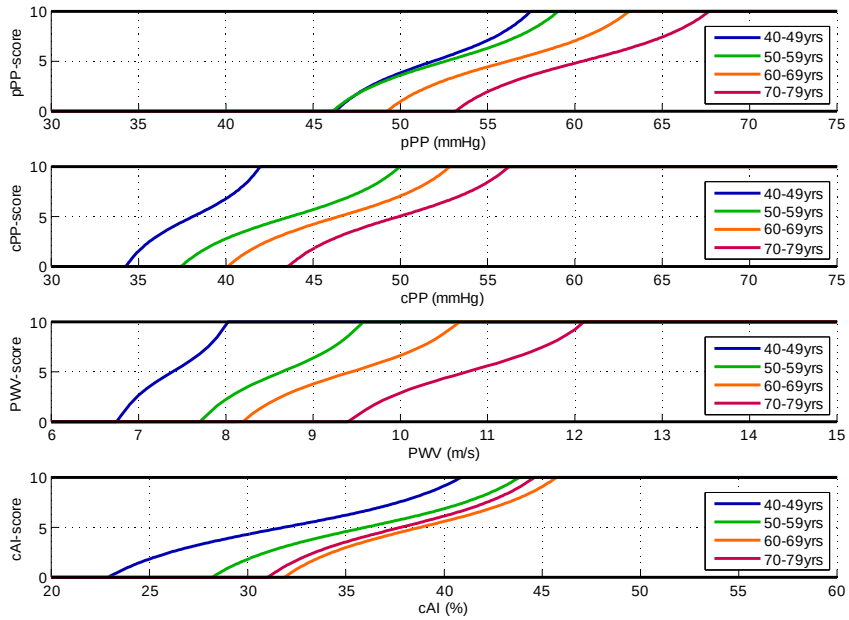


Figure 71: Calculated score ramps for pPP, cPP, PWV and cAI: categorized by age. Uncorrected unisex reference values calculated by reference value calculation rule 2 are used.

pPP and cPP: The pPP-score ramp is shifted to higher pPP input values for each decade of age. However, there are only slight differences between age group 1 and age group 2. In contrast, cPP-score ramps are located at lower pressure levels as pPP-score ramps. Contrary to pPP score ramps, there is a markedly difference between the cPP-score ramp of age group 1 and age group 2. For pPP and cPP, score ramps were strongly overlapped between age groups. The slope of the score ramps becomes less steep with increasing age due to a wider range of 'elevated' values for older subjects.

PWV: showed similar results compared to pPP and cPP. However, the distance between the PWV-score ramps of each age group is higher than found for pPP and cPP and there is also a shift to higher PWV input values for each decade of age.

cAI: Score ramps calculated for cAI are different compared to pPP, cPP and PWV. For cAI there was only a shift to higher cAI values from age group 1 to age group 2, and age group 2 to age group 3. For age group 3 to age group 4, there was a shift to lower cAI input values due to a decrease of reference values for age group 4.

Gender differences: The score calculation behavior differs depending on gender due to different reference values for male and female subjects. Therefore score ramps are shifted to lower or higher values depending on gender as defined by the pool of reference values.

Example based on PWV: Figure 72 shows the age dependency of score results for a specific measured PWV value of 9 m/s. It is shown that for older subjects it is acceptable to have a higher PWV value as for younger subjects.

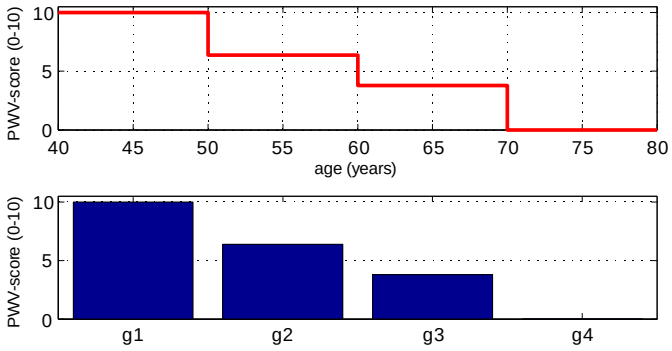


Figure 72: Course of the PWV-score for a measured PWV input value of 9 m/s. The top graphic shows how the PWV-score decreases for an increase of age. The bottom graphic shows the score results expressed as bars categorized by four different age categories (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Uncorrected unisex reference values calculated by reference calculation rule 2 are used.

3.3.4.2. Confounder dependency

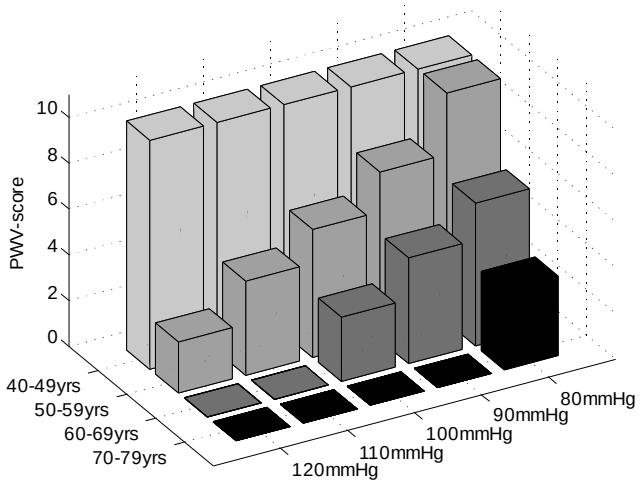


Figure 73: Calculated scores for a measured PWV input value of 9 m/s: categorized by four different age categories and five different mean arterial pressure levels. Uncorrected unisex reference values calculated by reference calculation rule 2 are used.

In addition to the age-dependency of the score calculation, there is a confounder-dependency of the score calculation. The FLSS was setup for the confounder-correction of PWV (for MAP) and cAI (for HR and body height). Figure 73 and Figure 74 show examples for the age- and confounder-dependencies of the score calculation for these variables.

PWV depending on MAP: Figure 73 shows an example of different score results for PWV depending on age and MAP. The bars indicate the resulting scores for a measured PWV of 9 m/s. Results are categorized by each defined age group and five different MAP levels. This example shows that scores decrease for an increase in MAP to account for the impact of blood pressure on PWV. This dependency is described by the calculated regression equations.

Gender differences: The impact of the confounder correction on score results is gender-dependent because regression lines differ between male and female subjects.

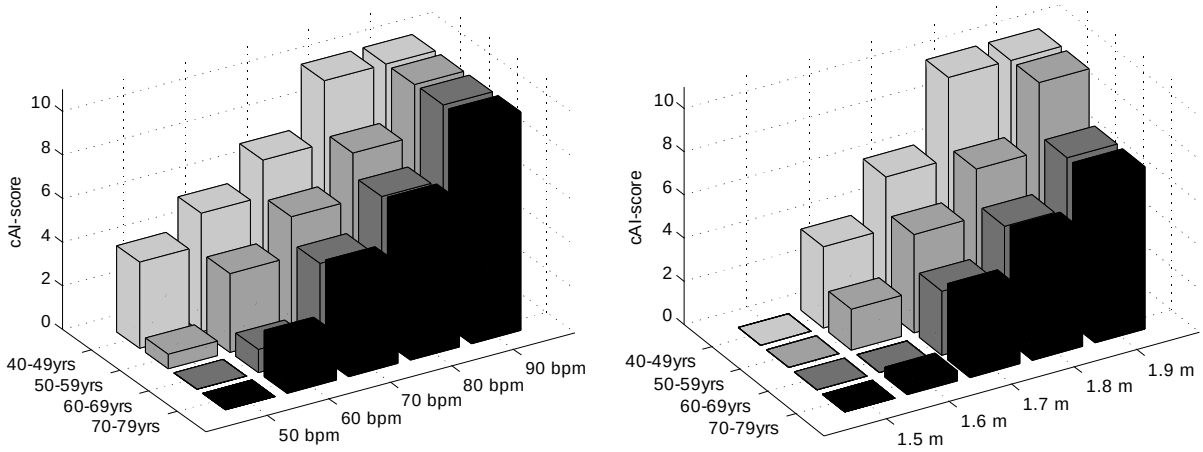


Figure 74: Calculated scores for a measured cAI of 35%. The left figure shows the HR-dependency of the score calculation (categorized by four different age categories and five different HR values; a constant value of 1.7 m for body height was used). The right figure shows the body height-dependency of the score calculation (categorized by four different age categories and five different body height values; a constant value of 65 bpm for HR was used). Uncorrected unisex reference values calculated by reference calculation rule 2 are used.

cAI depending on HR and body height: Figure 74 (left graphic) shows an example of different score results for cAI depending on age and HR. Figure 74 (right graphic) shows an example of different score results for cAI depending on age and body height. The bars indicate the resulting scores for a measured cAI of 35%. This example shows that scores decrease for a decrease in HR or body height. It is also shown that score results for age group 4 are slightly higher as for age group 3. This results due to lower reference values for age group 4 as for age group 3.

3.4. Discussion

3.4.1. Arterial stiffness as input information

Arterial stiffness cannot be readily measured non-invasively but it can be estimated by surrogate variables such as pPP, cPP, PWV and cAI. Hence, surrogate variables of arterial stiffness can be measured easily and used to assess the effects of arterial stiffness (Laurent et al., 2006). As described in Chapter 2, the present scoring concept and scoring technique is based on input variables which reflect the physiological or pathophysiological change of a variable with age. However, this investigation shows that surrogate variables of arterial stiffness can be used as input information for the scoring concept. Furthermore, it was shown that each surrogate variable is strongly age-dependent. These findings agree with findings of Franklin et al. (1997), Benetos et al. (2002), Mitchell et al. (2004), McEniery et al. (2005) and Mattace-Raso et al. (2010). However, to investigate the use of the scoring system based on surrogate variables of arterial stiffness it is necessary to investigate the impact of risk factors on score results. This is shown in Chapter 4.

3.4.1.1. Interpretation of score results

Figure 75 shows the scoring concept (see Section 2.2.1) adapted for surrogate variables of arterial stiffness. For each age group a pair of reference values ‘normal’ and ‘high’ defines a region of normal, elevated and high values. Hence, calculated scores (0 to 10) gives abstracted information on how much a surrogate variable of arterial stiffness has increased compared to normal levels.

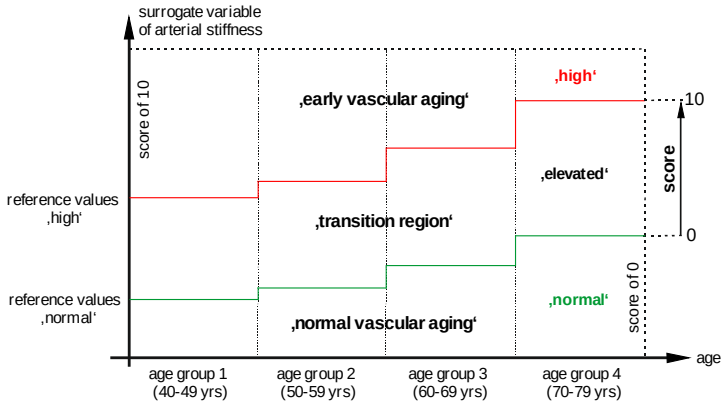


Figure 75: Interpretation of score results. For each surrogate variable of arterial stiffness a pair of reference values (‘normal’ and ‘high’) were defined.

Further interpretation: The concept of normal and early vascular aging differs between the physiological course of a variable with age (normal vascular aging) and a pathophysiological course of a variable with age (early vascular aging) (see Section 1.5.2). Therefore the region of ‘normal’ values (score of 0) can be interpreted as region of normal vascular aging and the region of high values (score of 10) can be interpreted as region of early vascular aging (see Figure 75). The region with ‘elevated’ values can be interpreted as transition region (score greater 0 and lower 10) between. In conclusion, scores based on surrogate variables of arterial stiffness can be interpreted as a measure of early

vascular aging but it is important to underline that calculated scores do not give information on a specific vascular age or biological age.

3.4.2. Reference group

The FLSS calculates scores in relation to reference values for each surrogate variable of arterial stiffness. This pool of reference values provides information for 'normal' and 'high' values for each variable, age category and gender category. Unfortunately, no guidelines were available which provide this information (see Section 3.1.3). Therefore the presented setup procedure was used to extract a reference group and to calculate reference values based on this reference group.

3.4.2.1. Filter criteria compared with guidelines

Reference values were calculated based on a highly selected reference group. Filter criteria were defined (see Section 3.2.1.2) to extract the reference group from the ACCT data. Table 1 and Table 2 compares the defined filter criteria with the official guidelines for each filter variable (TC, LDL cholesterol, HDL cholesterol, TG, BMI, SBP and DBP).

Table 1: Filter criteria compared with cholesterol guidelines. This table compares the used filter criteria for TC, LDL cholesterol, HDL cholesterol and TG with the ATP III classification (NCEP, 2002).

filter criterion	ranges	interpretation
TC < 6.2 mmol/l	< 5.0 mmol/l	desirable
	5.0 - 6.19 mmol/l	borderline high
	≥ 6.2 mmol/l	high
LDL < 4.1 mmol/l	< 2.60 mmol/l	optimal
	2.60 - 3.29 mmol/l	near optimal/above optimal
	3.30 - 4.09 mmol/l	borderline high
	4.10 - 4.89 mmol/l	high
	≥ 4.9 mmol/l	very high
HDL ≥ 1.0 mmol/l	≥ 1.55 mmol/l	high
	1.03 - 1.54 mmol/l	medium
	< 1.03 mmol/l	low
TG < 2.26 mmol/l	< 1.69	normal
	1.70 - 2.25 mmol/l	borderline high
	2.26 - 5.65 mmol/l	high
	≥ 5.65 mmol/l	very high

This comparison shows that only subjects who had normal or borderline normal values for each filter variable (TC, LDL, HDL, TG, BMI, SBP and DBP) were included to the reference group. Subjects with high values were excluded. That means the reference group reflects the distribution of surrogate variables of arterial stiffness (pPP, cPP, PWV and cAI) for subjects who had normal or borderline normal values for each filter variable at the time of measurement.

Table 2: Filter criteria compared with body weight and blood pressure guidelines. This table compares the used filter criterion for body weight (BMI) with the BMI classification of the WHO (WHO, 2011b). Further this table compares the used filter criteria for blood pressure (SBP and DBP) with the European guidelines for the management of arterial hypertension (Mancia, 2007).

filter criterion	ranges		interpretation
BMI < 30 kg/m^2	< 18.5		underweight
	18.50 - 24.99		normal
	25.00 - 29.99		overweight
	≥ 30		obese
	SBP	DBP	
SBP < 140 mmHg	< 120	< 80	optimal
	120 - 129	80 - 84	normal
DBP < 90mmHg	130 - 139	85 - 89	high normal
	140 - 159	90 - 99	grade 1 hypertension
	160 - 179	100 - 109	grade 2 hypertension
	≥ 180	≥ 110	grade 3 hypertension

3.4.2.2. Reference group versus normal group

It was shown that the impact of filter criteria on the distribution of surrogate variables of arterial stiffness is very strong. To investigate this fact, an additional normal group was extracted from the ACCT data to show the impact of strict filter criteria. Therefore a normal group was extracted under the use of stricter filter criteria compared to the reference group. That is, only subjects with normal values for each filter variable (no subjects with borderline normal values) were accepted. Following filter criteria were used: *subjects with TC ≥ 5mmol/l, LDL cholesterol ≥ 2.6mmol/l, HDL cholesterol < 1.55mmol/l, TG ≥ 1.69mmol/l, BMI ≥ 25kg/m^2, peripheral SBP ≥ 130mmHg, peripheral DBP ≥ 85mmHg and diabetes mellitus were excluded. Current smokers, past smokers and subjects receiving any medication were also excluded.* Following figure shows the comparison of PWV values in the reference group with PWV values in the normal group:

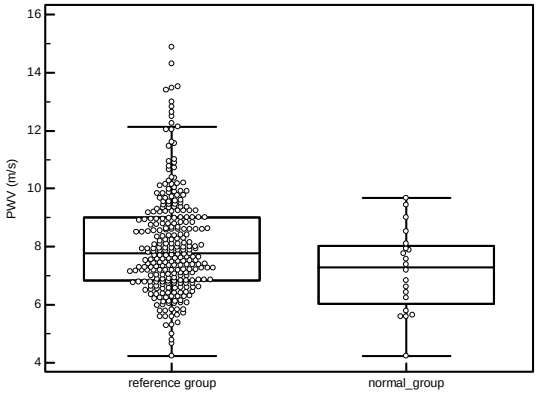


Figure 76: Comparison of reference group with normal group. Box-whisker-plot of reference group data (285 subjects) and normal group data (20 subjects) for PWV.

The normal group had significantly ($p<0.05$) lower PWV values compared to the reference group. Furthermore, only 20 subjects (normal group) fulfilled the strict filter criteria. Therefore, it is obvious that the definition of adequate filter criteria is an important point for the extraction of reference values which define the 'normal' state. The advantage of the extraction of a reference group with subjects who had normal or borderline normal values for each filter variable is that this group contains information about the normal and the borderline state of a variable.

3.4.2.3. Uncertainties for gender-specific subgroups

It is important to note that the number of subjects varies between surrogate variables due to the fact that not every dataset was complete. Furthermore, the separation of the reference group into age and gender-specific subgroups leads to a low number of subjects for some subgroups. For example, male subjects in age group 1 and female subjects in age group 4. Table 3 shows the number of subjects per subgroup. It is clear that results for gender-specific subgroups with a low number of subjects have higher uncertainties than subgroups with a higher number of subjects.

Table 3: Number of subjects per subgroup calculated for the reference group. Results are categorized for age and gender. Subgroups with a number of subjects lower than 20 are marked red.

age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP	M+F	n=50	n=62	n=106	n=59
	M	n=16	n=28	n=43	n=39
	F	n=34	n=34	n=63	n=20
cPP	M+F	n=46	n=46	n=100	n=54
	M	n=13	n=20	n=40	n=38
	F	n=33	n=26	n=60	n=16
PWV	M+F	n=50	n=61	n=105	n=57
	M	n=16	n=27	n=43	n=39
	F	n=34	n=34	n=62	n=18
cAI	M+F	n=50	n=57	n=106	n=59
	M	n=16	n=26	n=43	n=39
	F	n=34	n=31	n=63	n=20

3.4.2.4. Reference group results compared with literature

Results of pPP, cPP and cAI calculated for the reference group were compared with McEniery et al. (2005) and results of PWV were compared with Mattace-Raso et al. (2010).

3.4.2.4.1. pPP, cPP and cAI

McEniery et al. (2005) used also a cohort from the ACCT study. However, this cohort was not the same as used for the extraction of the reference group. McEniery et al. (2005) extracted in total 4001 subjects by following filter criteria from the ACCT cohort (10.096 subjects): peripheral *SBP < 140 mmHg, peripheral DBP < 90 mmHg, serum cholesterol < 6.5 mmol/l, no renal disease, no CVD and no medication intake.* In fact, the extracted reference cohort presented by McEniery et al. (2005) consisted of much more subjects (4001 subjects) compared to the extracted reference group for the FLSS (285 subjects).

Figure 77 shows the calculated mean values of pPP and cPP of the reference group categorized for male and female subjects compared with the mean values calculated by McEniery et al. (2005). A similar standard deviation for each age group was found. Subgroups with a number of subjects lower than 20 subjects were signed with a star (*) and were excluded from interpretation.

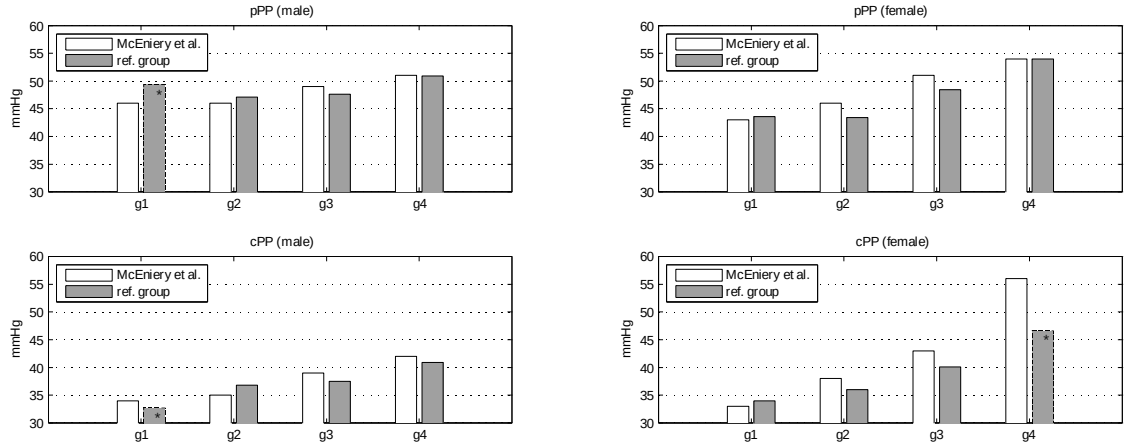


Figure 77: Comparison of results for pPP and cPP (reference group) with McEniery et al. (2005). Bars indicate the mean for each subgroup. Bars signed with a star (*) indicate that lower than 20 subjects were in the subgroup. Plots are separated for each variable and gender. Bars are separated for age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

pPP: Mean values presented by McEniery et al. (2005) indicate that pPP increases predominantly for male subjects over the age of 60 years and for female subjects over the age of 50 years. Franklin et al. (1997) showed that pPP calculated for normotensive and hypertensive subjects increases predominantly after the age of 50 years. In contrast, results calculated based on the reference group indicate that pPP increases predominantly over the age of 60 years for male and female subjects. The difference between these results could be explained by the fact that different filter criteria were used. Therefore, it could be hypothesized that strict filter criteria (reference group) extract subjects with a tendency to be healthier and therefore the increase of pPP values occurs in later life.

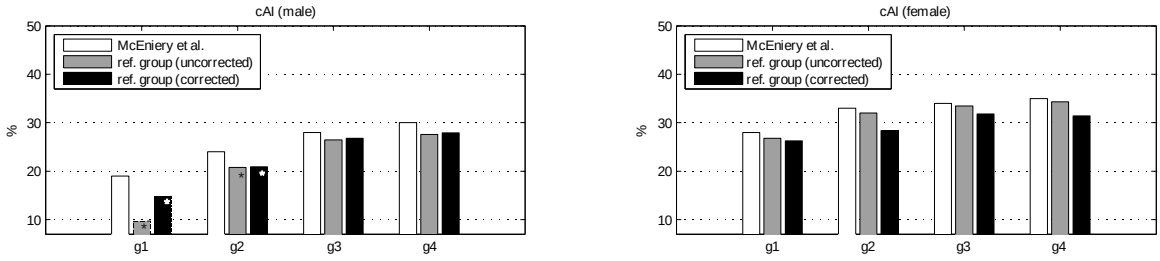


Figure 78: Comparison of results for cAI with results for cAI (confounder-corrected) and McEniery et al. (2005). Bars indicate the mean for each subgroup. White bars indicate results presented by McEniery et al. (2005). Grey bars indicate results calculated by the uncorrected reference group data. Black bars indicate results calculated by the confounder-corrected (HR and body height) reference group data. Bars signed with a star (*) indicate that lower than 20 subjects were in the subgroup. Plots are separated for male and female subjects. Bars are separated for age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

cPP: In most subgroups cPP was lower compared to results of McEniery et al (2005). Furthermore, McEniery et al. (2005) found a much stronger increase of cPP values for female subjects with age. However, this could be a further result of strict filter criteria because smokers were not excluded for

the study of McEniery et al. (2005). It was shown that smoking leads to an increase in total peripheral resistance (Omvik, 1996) and therefore it could be further hypothesized that this leads to an increase in wave reflections and cPP is strongly influenced by wave reflections.

Figure 78 shows the calculated mean values of cAI calculated by the uncorrected reference group compared with the mean values calculated by the corrected reference group (corrected for HR and body height) and with the mean values calculated by McEniery et al. (2005).

cAI: McEniery et al. (2005) found higher cAI values for male and female subjects. This could be a further result of different filter criteria. The cAI values were lower for female subjects and higher for male subjects after confounder-correction. However, the impact of the confounder-correction was much higher for female than for male subjects. This is predominantly an effect of the correction for body height because female subjects are tendentially smaller than male subjects.

3.4.2.4.2. Pulse wave velocity

Results for PWV were compared with PWV normal values calculated by Mattace-Raso et al. (2010). Figure 45 shows a box-whisker-plot of the used data calculated by Mattace-Raso et al. (2010). However, Mattace-Raso et al. (2010) used a cohort of 16 867 subjects measured from 13 different centers across Europe. Out of these, 1455 subjects were extracted. This subgroup was highly selected by following filter criteria: *peripheral SBP < 130 mmHg, peripheral DBP < 85 mmHg, no CVD, no current treatments, no diabetes mellitus, no smoking and no dyslipidaemia (TC > 5 mmol/l, HDL cholesterol < 1.0 mmol/l for men and HDL cholesterol < 1.2 mmol/l for women, LDL cholesterol > 3.0 mmol/l or TG > 1.7 mmol/l).* Compared with guidelines (see Table 1 and Table 2) Mattace-Raso et al. (2010) extracted only subjects with normal values for each filter variable.

Figure 79 shows the calculated mean values of PWV calculated for the uncorrected reference group compared with the mean values calculated for the corrected reference group (corrected for MAP) and the mean values calculated by Mattace-Raso et al. (2010).

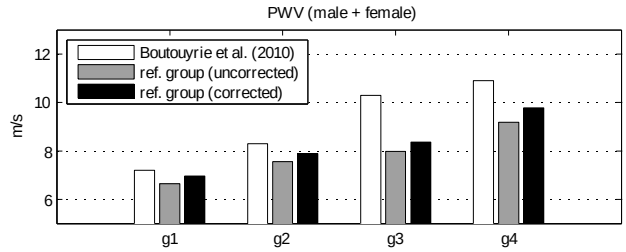


Figure 79: Comparison of results for PWV with results for PWV (confounder-corrected) and Mattace-Raso et al. (2010). Bars indicate the mean for each subgroup. White bars indicate results presented by Mattace-Raso et al. (2010). Grey bars indicate results calculated by the uncorrected reference group data. Black bars indicate results calculated by the confounder-corrected (MAP) reference group data. Bars are separated for age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

Mattace-Raso et al. (2010) found higher PWV values compared to the results of the reference group although Mattace-Raso et al. (2010) excluded only subjects with normal values for each filter variable.

The difference in results could be due to the fact that Mattace-Raso et al. (2010) used different devices and further different algorithms for the calculation of the 'foot of the wave'. As shown in Millasseau et al. (2005) different algorithms can lead to differences in calculated PWV values of 5 to 15 %. Therefore Mattace-Raso et al. (2010) standardized the calculation of PWV. Furthermore, the measurement of the distance (Δx) (see (35)) on the body surface had to be standardized by Mattace-Raso et al. (2010). The process of standardizing and the used equations can be found in Mattace-Raso et al. (2010, p2-4). In conclusion, the resulted differences between the reference group and Mattace-Raso et al. (2010) could be explained by different devices, algorithms and standardizing procedures.

3.4.3. Reference values

Reference values were calculated based on the extracted reference group. As described in Section 3.4.2, the reference group reflects the distribution of surrogate variables of arterial stiffness for subjects who had normal or borderline normal values for the used filter variables (TC, LDL cholesterol, HDL cholesterol, TG, BMI, peripheral SBP and peripheral DBP) at the time of measurement. Therefore, it was assumed that the distribution of each surrogate variable of arterial stiffness can be used to calculate a reference value which defines the 'normal' state and a reference value which defines the 'high' state. Therefore four different reference value calculation rules (see Section 3.2.1.3) were defined based on statistical parameters.

3.4.3.1. Definition of reference values

For the definition of the reference value 'normal' it is common to use the mean or median of a group of 'healthy' subjects but there was no standardized procedure to define a reference value 'high' found in the literature. The definition of an elevated interval between these two reference values is important because single cut-off values do not account for the smooth transition between normal and high values. Due to this fact it was assumed that the reference group reflects the distribution of normal and borderline normal subjects. That means the area around the mean and the median of a distribution can be used for the definition of the reference value 'normal' and the outside area of the distribution can be used for the definition of a reference value 'high'. This is conceptually shown in the following figure:

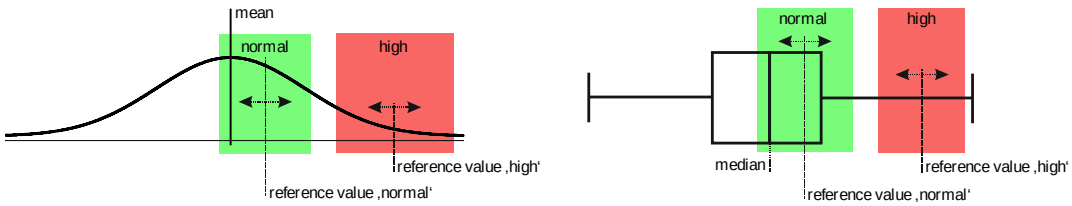


Figure 80: Distribution-based definition of reference values. The left graphic demonstrate the definition areas (normal and high) for reference values based on an ideal standard normal distribution. The right graphic demonstrate the definition areas (normal and high) for reference values based on statistical parameters presented by a box-whisker-plot.

3.4.3.1.1. Limitations and simplifications

This concept has some limitations and simplifications: As shown in Figure 80 there is a wide range for the definition of reference values within the definition areas. Hence, in a specific range the definition

of the reference values is arbitrary. In addition, the lower the reference value 'normal' is chosen the fewer subjects have a score of 0 and the lower the reference value 'high' is chosen the more subjects have a score of 10. To account for this problem, four different reference value calculation rules (see Figure 81) were defined and the effect on results was investigated. A further approach to address this problem could be the use of data of prospective cohort studies which reflect the individual risk for CVD events. That is, based on these data reference values could be defined based on the individual risk for having a CVD event.

A further limitation of this approach is that it is problematic to define reference values based on subgroups with a low number of subjects because the calculated statistical parameters based on these subgroups have significant uncertainties. It follows that the definition of reference values based on subgroups with a low number of subjects has also significant uncertainties.

Most biological variables do not show a standard normal distribution. However, some variables show a standard normal distribution after logarithmic transformation. In this investigation this was true for pPP, cPP and PWV. In conclusion, an ideal standard normal distribution was assumed and therefore this is a simplification of this approach.

As described in Chapter 2, calculated scores do not give information on cardiovascular risk. In addition this kind of definition of reference values has no relation to cardiovascular risk. To account for this problem the use of data of prospective cohort studies would be important too.

3.4.3.2. Comparison of reference value calculation rules

Figure 81 shows the defined reference value calculation rules 1-4. Details can be found in Section 3.2.1.3. The definition of the reference value calculation rules had following characteristics.

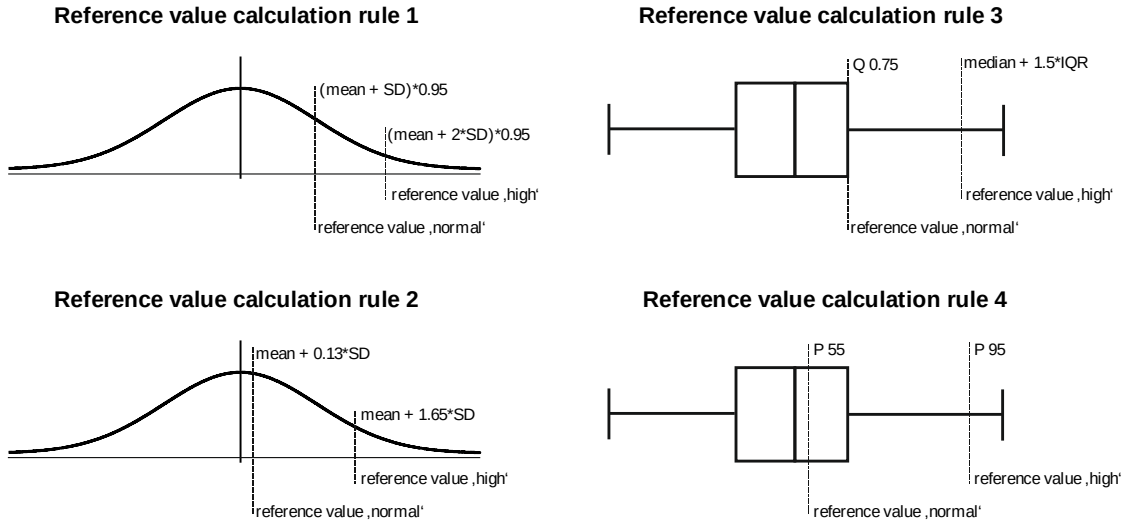


Figure 81: Interpretation of reference value calculation rules (1-4). The reference value calculation rules 1 and 2 are based on median and standard deviation. The reference value calculation rule 3 is based on median, interquartile range and the third quartile value (Q 0.75) and reference value calculation rule 4 is based on percentile value (P55, P95).

Definition of calculation rule 1: For the reference value 'normal' a relatively high value was defined compared to the calculation rule 2 and 4. This definition leads to greater proportion of subjects with a score of 0, a small group of subjects with scores between 0 and 10 and only a few subjects with a score of 10 in the reference group.

Definition of calculation rule 2 and 4: The reference value 'normal' was chosen nearby the mean and respectively the median of the reference group. For both rules it was defined that approximately 55% of the subjects of the reference group have a score of 0 and approximately 5% of the reference group have a score of 10. This definition was used to account for the fact that the reference group consists of subjects who had normal or borderline normal values for the used filter variables but no high values.

Definition of calculation rule 3: The calculation rule 3 was defined in such a way that this definition leads to more subjects with a score between 0 and 10 compared to calculation rule 1.

The comparison shows that there is a wide range for the calculation of reference values based on statistical parameters. Therefore a further investigation and validation of an adequate and standardized procedure would be important for a future use of the FLSS. However, Chapter 4 presents a further comparison of score results calculated by different calculation rules.

3.4.3.3. Characteristics of calculated reference values

Unisex reference values: Reference values (see Figure 60) for pPP, cPP and PWV increase steadily with age. An exception was pPP for calculation rule 2 and 4. This was due to the fact that pPP shows a plateau effect for younger subjects under the age of 60 years (see Section 3.4.2.4.1). In contrast, reference values calculated for cAI show a plateau effect for older subjects. This was due to the fact that cAI shows a plateau effect for subjects over the age of 60 years (see Figure 53).

Gender-specific reference values: The calculation of separated reference values for male and female subjects (see Figure 61 and Figure 62) was strongly affected by the impact of the low number of subjects for some gender-specific age groups (see Table 3). However, reference values calculated on statistical parameters such as mean and standard deviation (calculation rule 1 and 2) showed improved results compared to reference values calculated by statistical parameters such as median, interquartile range, quartile values and percentile values (calculation rule 3 and 4). That indicates that if the number of subjects is low it is of benefit to use calculation rule 1 or 2.

Confounder-corrected reference values: The calculation of reference values based on confounder-corrected data resulted higher PWV reference values for male and female subjects, higher cAI reference values for male subjects and lower cAI reference values for female subjects. This is a result of the confounder-correction of the reference group data. Therefore it is obvious that reference values were shifted in the same way to higher and lower values as the data were shifted due to the confounder-correction process.

Conclusion: In conclusion, best results for the calculation of reference values can be obtained by calculation rule 1 and 2 especially in the case of a low number of subjects. That is, reference values calculated based on mean and standard deviation are preferable.

3.4.3.4. Reference values compared with literature

pPP, cPP and cAI: No guidelines define age-separated normal values for pPP, cPP and cAI. However, some studies calculated mean or median values based on cohorts who can be considered as healthy. McEniery et al. (2005) presented mean values for pPP, cPP and cAI (see Figure 77 and Figure 78). However, it is unclear if mean or median values can be directly interpreted as normal values. That means if the mean or median value is used as the normal value approximately half of the reference cohort would have values higher than the normal value. In addition it is important to consider that the defined filter criteria have a significant effect on the results. However, mean values calculated by McEniery et al. (2005) were in most cases similar or lower compared to the calculated reference values 'normal'. In fact the difference depends on the used reference value calculation rule.

PWV: The following figure shows an overview for the suggested normal and reference values for PWV. It is obvious that reference values presented by Mancia et al. (2007) and Alecu et al. (2008) do not account for age. However, Mattace-Raso et al. (2010) considered the physiological increase of PWV with age. This study presented mean and median values for PWV calculated by a healthy cohort (see Figure 45). However, calculated reference values 'normal' for age group 1 and 2 tended to be similar and for age group 3 and 4 tended to be higher in comparison with the results of Mattace-Raso et al. (2010).

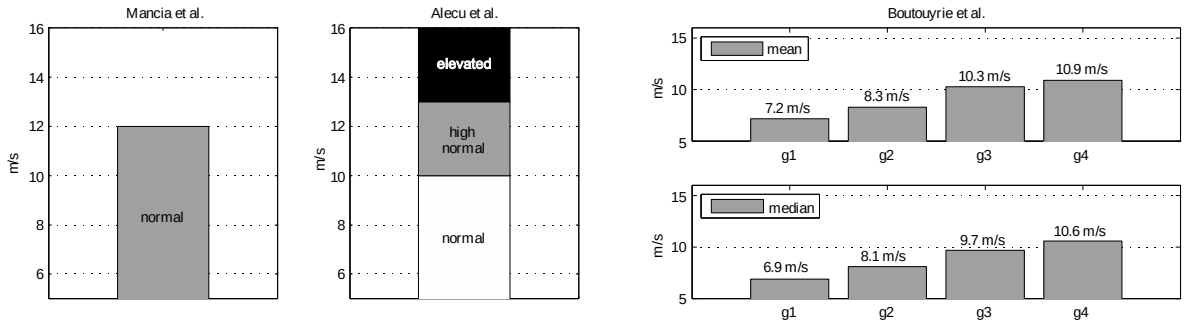


Figure 82: Comparison of normal and reference values for PWV. The left graphic shows a threshold value for PWV suggested by Mancia et al. (2007). The middle graphic shows two threshold values for normal, high normal and elevated values suggested by Alecu et al. (2008). The right graphics show the mean and the median values calculated for a normal value population (1455 subjects) presented by Mattace-Raso et al. (2010).

3.4.4. Confounder correction

The FLSS accounts for confounders. PWV is corrected for the impact of MAP and cAI is corrected for the impact of HR and body height. These relationships were estimated based on the ACCT data and extracted subgroups (reference group and reference group with included hypertensive subjects). However, this procedure had an important limitation (described below).

3.4.4.1. Intra-individual vs. inter-individual relationships

Calculated regression equations were calculated based on inter-individual data (ACCT data). Therefore, it is important to mention that there is a difference between the intra-individual and the inter-individual measured relationship between a variable and a confounder. For example, the intra-individual relationship of PWV has to be determined in the same subject at high and low blood pressure levels. Hence, repeated measurements have to be performed for each subject but the ACCT data didn't serve this information. Hence, calculated regression equations for PWV and blood pressure were calculated as inter-individual relationships between subgroups that having high or low blood pressure.

Impact on the confounder-correction of PWV: The disadvantage of inter-individual data is that for each subject only one measurement was performed. Furthermore, each subject had different blood pressure levels at the point of measurement. That means subjects with high blood pressure levels were included into the analysis (ACCT data and reference group with included hypertensive subjects) but long-term hypertension leads to structural changes of the arterial vessel wall (see also Alexander (1995)). The following figure shows the impact of a short-term and a long-term increase of blood pressure on PWV.

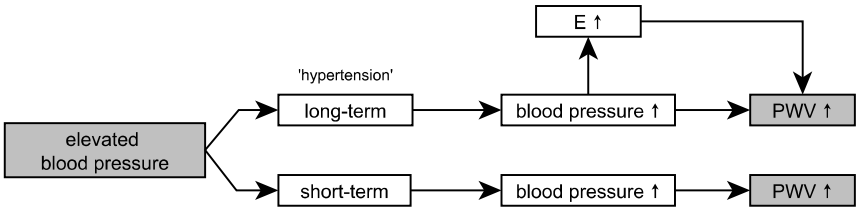


Figure 83: The impact of short-term and long-term elevated blood pressure on PWV. Short-term elevated blood pressure leads to a short-term increase of PWV. In contrast, a long-term elevated blood pressure (arterial hypertension) increases PWV and leads to a change in arterial structure (arterial wall elasticity decreases) which leads to a further increase of PWV.

Impact on the confounder-correction of cAI: Inter-individual data (ACCT data) were also used for the investigation of the relationship between cAI and confounders (HR and body height). Fortunately, body height is always an inter-individual factor and a change in HR doesn't lead to structural changes of the arterial vessel structure. Therefore, it is hypothesized that the impact of the use of inter-individual data instead of intra-individual data are reduced compared to PWV and blood pressure.

3.4.4.2. Comparison of calculated regression equations with literature

3.4.4.2.1. Pulse wave velocity

Inter-individual regression equations for PWV were calculated based on three different groups of data: ACCT data, reference group and reference group with included hypertensive subjects. Results are shown in Section 3.3.3.1. This investigation shows that PWV increases with increasing MAP levels and this relationship becomes more prominent with increasing age. A similar inter-individual increase of PWV with blood pressure was found in Mattace-Raso et al. (2010). Figure 84 shows the calculated

regression lines for different age categories. Mattace-Raso et al. (2010) showed that PWV at any age is linearly related to blood pressure. This agrees with the results of this present analysis.

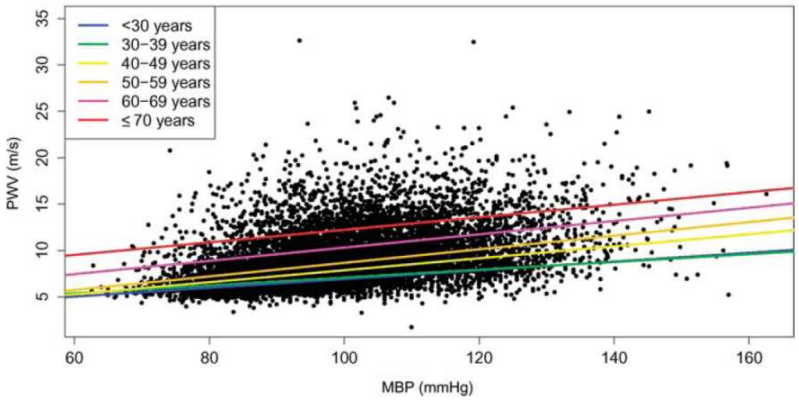


Figure 84: Relationship between PWV and MAP calculated on a large European cohort: categorized for decades of age (reproduced from Mattace-Raso et al., 2010).

ACCT data: Regression equations calculated based on the ACCT data (see Figure 64) showed an increase of the gradient of the regression line for each decade of age. Age group 3 and 4 showed a similar gradient. The same effect was shown in Mattace-Raso et al. (2010) (see Figure 84). The difference between male and female can be interpreted as marginal.

Reference group: Hypertensive subjects (peripheral SBP ≥ 140 mmHg and peripheral DBP ≥ 90 mmHg) were excluded for the extraction of the reference group. Due to this procedure only a few significant relationships between PWV and MAP were found for the reference group (see Figure 65). That means the inter-individual relationship between PWV and MAP can only be assessed adequately by including subjects with high blood pressure levels.

Reference group with included hypertensive subjects: This group resulted in similar relationships as calculated for the ACCT data (see Figure 66). Therefore regression equations calculated based on the ACCT data were used for the confounder correction in the FLSS.

Comparison with intra-individual data: There is a lack of information on the intra-individual relationships between PWV and MAP categorized by age. For instance, Carroll et al. (1991) investigated these relationships based on three subjects with dilated cardiomyopathy as shown in Figure 41. This investigation showed a similar increase of the gradient of the regression line with increasing age. However, the intra-individual relationships have to be further investigated.

PWV and HR: Lantelme et al. (2002) showed that PWV increases during cardiac pacing at different pacing frequencies. Contrary, only a weak relationship between PWV and HR was found based on the used inter-individual data (ACCT data). Therefore, it could be hypothesized that there is a difference between a short-term (intra-individual investigation) and a long-term (inter-individual investigation) effect.

3.4.4.2.2. Central augmentation index

Inter-individual regression equations for cAI were calculated based on two different groups of data (ACCT data and reference group). Results are shown in Section 3.3.3.2. This investigation shows that cAI decreases with increasing HR or body height. These results agree with the intra-individual relationship presented by Wilkinson et al. (2000) (see Figure 42) and McGrath et al. (2001) (see Figure 43).

ACCT data: The results for cAI depending on HR (see Figure 67) indicates that the relationship between cAI and HR is nearly unchanged over age. The relationship is slightly shifted to higher cAI values for male subjects with age. However, the negative gradient of the regression lines for cAI and body height decreases with age (see Figure 68). Therefore, it can be hypothesized that the effect of the travel distance on cAI is reduced with increasing age. This could be an effect of the increase of PWV with age.

Reference group: For unisex results, similar results were found as calculated for the ACCT data. For some gender-specific subgroups (see Figure 69 and Figure 70) results were not significant due to the low number of subjects. Therefore gender-specific results cannot be used for interpretation and for the confounder-correction in the FLSS.

3.4.4.3. Confounders of pPP and cPP

The variables pPP and cPP are predominately dependent on stroke volume but there was only incomplete data on stroke volume served by the ACCT data. Therefore these relationships were not investigated and corrected by the FLSS.

3.4.4.4. Confounder-correction of the reference group data

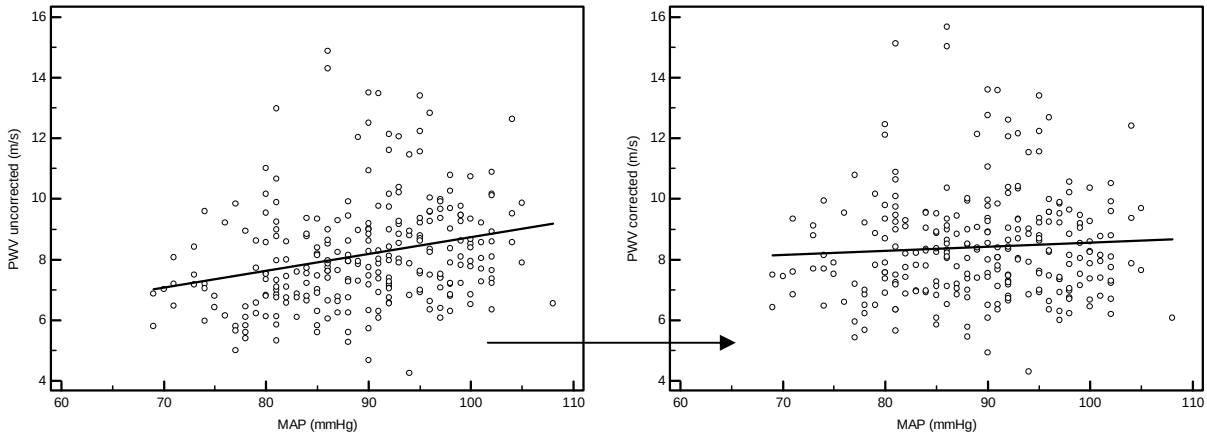


Figure 85: Confounder correction of PWV data (reference group). This figure shows the impact of the confounder-correction of the reference group data for PWV. The regression line is plotted for uncorrected PWV values (left figure) and for corrected PWV values (right figure).

The data of PWV and cAI of the reference group were corrected for the impact of confounders to calculate confounder-corrected reference values. Details can be found in Section 3.2.1.5. The

following figure shows the plotted data of PWV (reference group) before and after confounder-correction. It is shown that this procedure reduces the influence of blood pressure on the data.

3.5. Conclusions

Surrogate variables of arterial stiffness (pPP, cPP, PWV and cAI) can be measured easily and used as input information for the FLSS. For the practical setup of the FLSS it is necessary to use adequate external data (reference values and regressions equations). However, due to the lack of reference values for surrogate variables of arterial stiffness and further due to the lack of regression equations for the confounder-correction, external data were calculated based on data of the ACCT database (4643 subjects). Following main findings and main limitations can be summarized based on the present setup procedure and the present results from this investigation.

3.5.1. Main findings

Reference group: Based on the results of the extracted reference group it was shown that surrogate variables of arterial stiffness show a steady increase with age, although pPP showed a plateau effect for middle-aged subjects and cAI showed a plateau effect for elderly subjects. Mean values of pPP were higher than mean values of cPP. These findings agree predominantly with findings from the literature although some differences were found due to the use of different filter criteria and data pools. Additionally, it was shown that the adequate definition of filter criteria for the extraction of the reference group has a significant effect on results and further on the number of subjects per subgroups. Therefore some gender-specific results showed strong uncertainties due to a low number of subjects.

Reference values: Four different reference value calculation rules were defined and investigated to calculate reference values which define the normality and the abnormality for each surrogate variable separated for decades of age. It was shown that reference value calculation rule 1 and 3 lead to higher reference values for the normality of a surrogate variable of arterial stiffness than reference value calculation rule 2 and 4. It was also shown that calculation rule 2 is preferable if the number of subjects in a specific subgroup is low. Furthermore, reference values for pPP, cPP and PWV increased for most cases steadily with age and cAI showed for all cases a plateau effect for elderly subjects.

Confounder-correction: It was shown that regression equations calculated based on the whole ACCT data should be used for the assessment of the confounder-correction of PWV and cAI. Therefore, regression equations calculated based on the ACCT data were used for further investigations.

3.5.2. Main limitations and future work

The definition of reference values based on reference value calculation rules and respectively based on statistical parameters are in a specific range arbitrary and for a practical use of the FLSS based on surrogate variables of arterial stiffness a further validation is necessary. Furthermore, some results showed strong uncertainties due to a low number of subjects for some gender-specific subgroups.

Hence, further investigations should be predominantly focused on unisex-reference values and regression equations. Furthermore, an important limitation was the use of inter-individual data to assess the impact of confounders on PWV and cAI. Hence, the investigation of the impact of the use of inter-individual data instead of intra-individual data is important and requires further investigation.

4. Impact of risk factors

4.1. Introduction

For the use of surrogate variables of arterial stiffness as input information for the presented scoring technique it is important to investigate the impact of risk factors on score results. Literature showed that the progression of arterial stiffness can be negatively affected by a variety of lifestyle and disease factors such as smoking (Jatoi et al., 2007), obesity (Safar et al., 2006) or diabetes mellitus (Cruickshank et al., 2002). However, the impact of smoking, obesity and DM II on score results was investigated.

4.1.1. Risk factors and arterial stiffness

4.1.1.1. Impact of smoking

The use of tobacco is one of the most important causes of acute myocardial infarction (Teo et al., 2006). Various studies indicate a strong impact of smoking, passive smoking or smoking cessation on arterial stiffness: Mahmud and Feely (2003a, 2003b), Vlachopoulos et al. (2004a, 2004b), Jatoi et al. (2007) and Doonan et al. (2010). These studies indicate further that smoking leads to a notable increase in arterial stiffness. Jatoi et al. (2007) showed that current and ex-smokers had significantly higher PWV values and AI values compared with nonsmokers.

4.1.1.2. Impact of obesity

The impact of smoking on arterial stiffness is better investigated as the impact of obesity on arterial stiffness. However, Safar et al. (2006) concluded that individuals with obesity are likely to have an increase in aortic stiffness, independent of blood pressure levels, ethnicity and age. Safar et al. (2006) concluded further that the pathological mechanisms that link abdominal adiposity to stiffening are not fully understood. Zebekakis et al. (2005) used a wall-tracking ultrasound system to measure the properties of the carotid, femoral and brachial arteries and carotid-femoral PWV. This study showed that across a wide age range, the diameter and stiffness of muscular arteries increased with higher BMI.

4.1.1.3. Impact of diabetes mellitus

Cruickshank et al. (2002) demonstrated that PWV was greater in subjects with diabetes than in controls and that aortic PWV is a powerful independent predictor of mortality in diabetes samples. Salomaa et al. (1995) concluded that persons with non-insulin-dependent diabetes mellitus or borderline glucose intolerance have stiffer arteries than their counterparts with normal glucose tolerance and that the decreased elasticity is independent of artery wall thickness. Lacy et al. (2004) demonstrated that PP and PWV are increased in subjects with diabetes mellitus, but this is not

associated with increased cAI. Hence, this study shows that increased PWV is not associated with elevated cAI in patients with diabetes mellitus.

4.1.2. Investigation of the impact of risk factors on score results

Risk factors such as smoking, obesity and DM II have an impact on arterial stiffness and further on surrogate variables of arterial stiffness. Thus, it was hypothesized that is possible to investigate the impact of risk factors on score results (see Sections 1.7.3.1 and 1.7.4). Therefore, it was investigated how much the score distribution is shifted to higher score and respectively lower score values for a group of subjects under risk exposure. Based on this concept it is also possible to investigate which surrogate variable of arterial stiffness respond more sensitively to a specific risk factor.

However, other studies have suggested that the role of surrogate variables as markers of elevated arterial stiffness varies with age. McEniery et al. (2005) hypothesized that cAI is a better predictor in younger and PWV in older subjects. To investigate these associations score results were used to check if surrogate variables have a different performance as classification variables between middle-aged (40-59 years) and elderly subjects (60-79 years).

4.2. Methods

The impact of three different risk factors on score results was investigated. Therefore, three risk groups (smoker group, obesity group, and DM II group) were extracted from the ACCT data additional to the reference group. Filter criteria were defined for each risk group. Score results for pPP, cPP, PWV and cAI were then calculated for each risk group and further for the reference group. Subsequently, the risk group results were compared in relation to the reference group results.

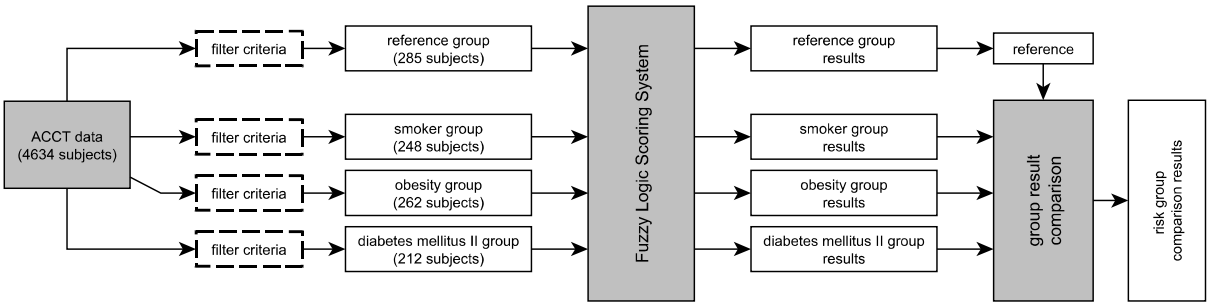


Figure 86: Risk group comparison procedure. Three risk groups were extracted from the ACCT data and their score results were compared with the score results of the reference group.

4.2.1. Risk group extraction procedure

Three risk groups were extracted from the ACCT data by using filter criteria (see Figure 86). The extracted risk groups were then divided into four age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Following filter criteria were used:

Filter criteria for smoker group: *Non-smokers and past-smokers were excluded. Additionally, obese subjects (BMI ≥ 30 kg/m^2) or subjects with diabetes mellitus (I or II) were excluded. Subjects receiving any vasoactive medication were also excluded.*

Filter criteria for obesity group: *Subjects with a BMI < 30 kg/m^2 were excluded. Additionally, smokers, past smokers or subjects with diabetes mellitus (I or II) were excluded. Subjects receiving any vasoactive medication were also excluded.*

Filter criteria for DM II group: *Subjects without diabetes mellitus or with diabetes mellitus I were excluded. Smokers, past smokers or obese subjects (BMI ≥ 30 kg/m^2) were excluded.*

No subject of the smoker group, obesity group or DM II group had overt CVD at time of measurement.

4.2.2. Group result calculation procedure

A score for each variable (pPP, cPP, PWV and cAI) was calculated based on each subject. This procedure was done separately for each risk group (smoker group, obesity group, DM II group) and the reference group as shown in Figure 86. Therefore each group was separated into subgroups as shown in Figure 87. Subgroups were defined by gender categories (male, female and unisex) and age groups (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years). Results were calculated for each subgroup by the FLSS separately. Furthermore, for each subject in a subgroup the score results were also calculated separately. These calculation procedure yields a data cluster called ‘group results’. This data cluster can be further used for the analysis of the results.

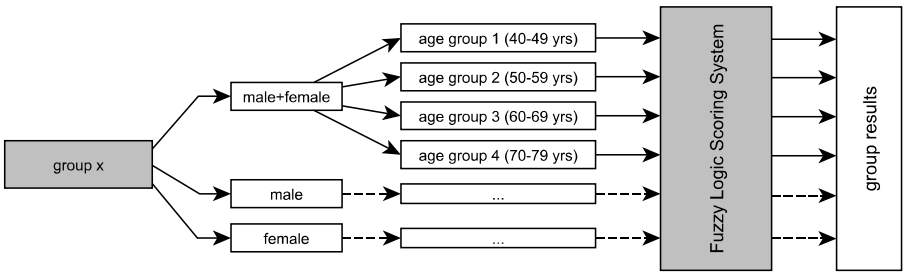


Figure 87: Flow chart of the group result calculation procedure. Each group was separated into subgroups. Results of each subgroup were clustered into the data cluster ‘group results’.

4.2.2.1. Settings of the FLSS

Calculated group results are dependent on the used settings of the FLSS. That is, different pools of reference values and further settings for confounder-correction are available. In conclusion, for this investigation several settings were used and compared with each other. Following settings are basically available as shown in the following figure:

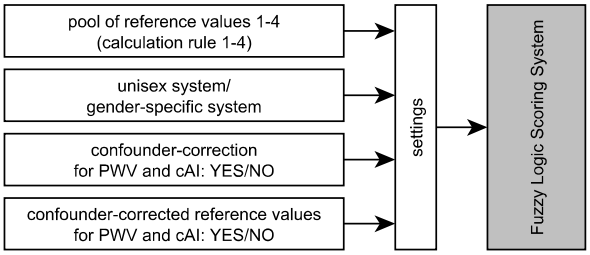


Figure 88: Setting options for the FLSS.

Pool of reference values: Four different reference value calculation rules (see Section 3.2.1.3) were defined. Hence, four different pools of reference values are available for the score calculation.

Unisex or gender-separated reference values and regression equations: Reference values and regression equations were calculated based on unisex data and gender-separated (male/female) data. Hence, the FLSS can be used as unisex or gender-specific system.

Confounder-correction of the score calculation of PWV and cAI: The use of the confounder-correction (RS-CC-system) can be enabled or disabled. Hence, the FLSS can be used with or without the confounder-correction of PWV and cAI. However, regression lines calculated based on the ACCT data were used for the confounder-correction of PWV and cAI in this investigation.

Confounder-corrected reference values: Reference values for PWV and cAI were calculated based on data of the reference group and additionally based on data of the confounder-corrected reference group. Hence, uncorrected or confounder-corrected reference values for PWV and cAI can be used for the FLSS.

4.2.3. Group result comparison procedure

The impact of different settings of the FLSS was investigated. Furthermore, calculated risk group results were compared in relation to the reference group results as shown in Figure 86. Therefore two different procedures were used:

4.2.3.1. Plot of group and subgroup data

Group and subgroup results were plotted as cumulative distribution plots. This procedure was used to visualize the score distribution for each group and subgroup. Hence, differences between groups and subgroups can be visualized. Figure 89 shows an example of a cumulative distribution plot and the interpretation of the plot.

The distribution of the data is plotted as cumulative distribution step function. The x-axis displays the score range from 0 to 10. The y-axis displays the fraction of subjects from 0 to 1 (0% of subjects to 100% of subjects). The plotted line shows the fraction of subjects that have a score less than a specific score value x. Therefore the fraction of subjects with normal values (score of 0) can be seen at score is 0 (A). The fraction of subjects with ‘high’ values (score of 10) can be calculated by subtracting the y-

value at score is 10 from 1 (100% of subjects) (C). And the fraction of subjects with 'elevated' values (score greater 0 and lower 10) can be calculated by subtracting the y-value at score is 0 from the y-value at score is 10 (B).

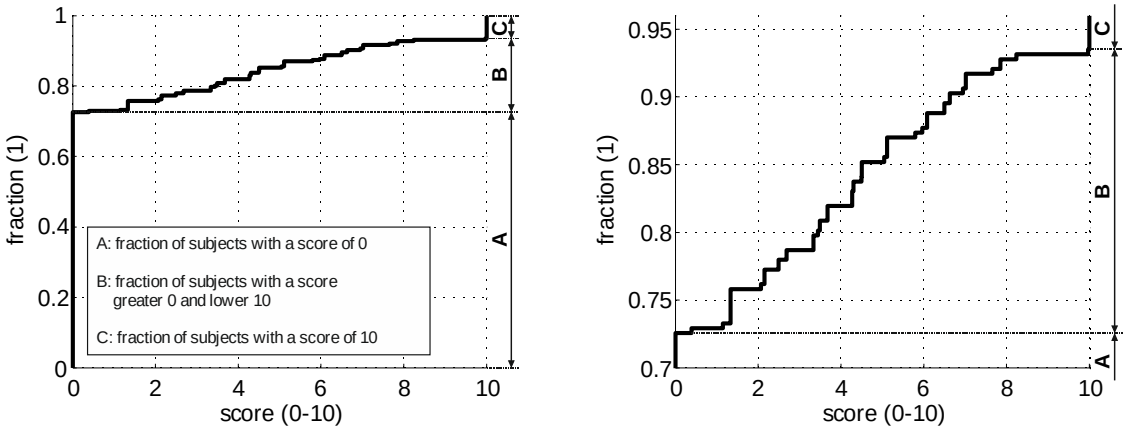


Figure 89: Interpretation of the cumulative distribution plot of score results. The left figure shows the cumulative distribution plot of score data for the whole fraction range. The right figure shows a zoom of the left plot. The letters A, B and C indicates the fraction of subjects.

4.2.3.2. Group and subgroup comparison with reference group

The second procedure was used to compare the risk group results in relation to the reference group results. However, in a group of subjects with risk factor exposure there is a fractional shift of score results to higher values. The fractional shift due to risk factor exposure can be calculated as difference in the AUC between the reference group and the risk group as shown in Figure 90 (left graphic). Therefore the AUC of a risk group is subtracted from the AUC of the reference group.

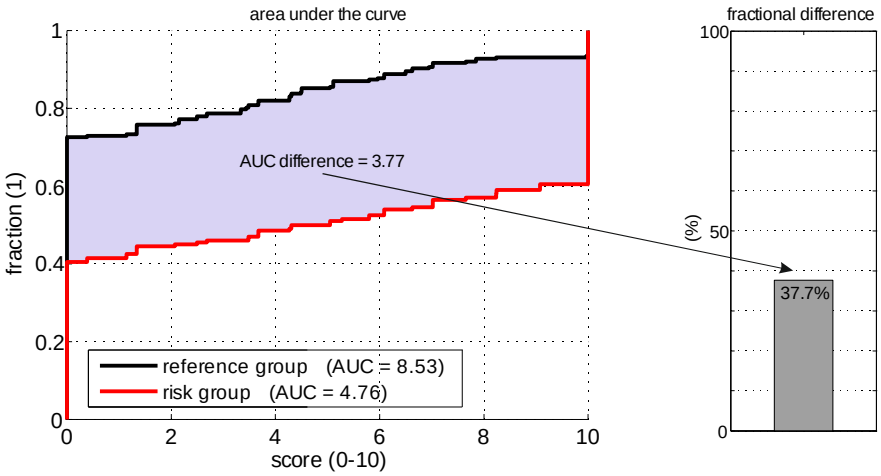


Figure 90: Calculation of fractional differences. Fractional differences are calculated as the difference in the AUC between the reference group and the risk group (see left Figure). Further the fractional difference is defined as the difference in the AUC multiplied by 10 (see right Figure).

The AUC is calculated as shown in the following equation whereas the variable n is the number of data points of the cumulative distribution function, the variable s is the score value and the variable f is the fraction of subjects.

$$AUC = \sum_{k=1}^{\frac{n}{2}} \left(s_{(2\cdot k)} - s_{(2\cdot k-1)}\right) \cdot f_{(2\cdot k)} \qquad \textbf{(56)}$$

This procedure was done for each risk group and respectively for each subgroup of each risk group. In conclusion, this procedure can be seen as a measure of the fractional shift (resulted AUC difference) to higher score results for a risk group in relation to the reference group. Therefore the AUC difference is multiplied by 10 and called as fractional difference as shown in Figure 90 (right graphic).

4.3. Results

The results presented in the following sections give information on the impact of risk factors on score results. Furthermore, the impact of different settings of the FLSS (reference calculation rules and confounder-correction) is shown. A detailed summary statistic of each extracted risk group (smoker group, obesity group and DM II group) can be found in the appendix (see Table 16-Table 18). The smoker group consisted of 248 subjects, the obesity group consisted of 262 subjects and the DM II group consisted of 212 subjects.

4.3.1. Comparison of reference value calculation rules

The effect of different reference value calculation rules are shown in Figure 91 and Figure 92. Therefore, cumulative distribution plots for each variable and risk group were calculated. Each subplot displays the resulting cumulative distribution calculated by the reference value calculation rules 1-4. *Following settings were used for this investigation:* ***Group data:*** *male + female subjects;* ***reference values:*** *unisex reference values, calculated by rule 1 (blue lines), calculated by rule 2 (red lines), calculated by rule 3 (magenta lines), calculated by rule 4 (green lines);* ***confounder correction:*** *no, uncorrected reference values for cAI and PWV.*

Comparison of reference value calculation rules: Results differed between calculation rules predominantly for low score results (score of 0) than for high score results (score of 10). It was also shown that the reference value calculation rule 1 and 3 led to similar results for pPP, cPP, PWV and cAI for each group of data. Although differences between calculation rule 1 and 3 were higher for pPP and cPP. Calculation rule 2 and calculation rule 4 showed similar results for each group of data as well. In comparison calculation rule 2 and 4 led to approximately 10% less subjects with a score of 0 compared to calculation rule 1 and 3.

Impact of risk factors: Smoking, obesity and DM II led to a notable shift to higher score results for pPP, cPP and PWV compared to the reference group. However, DM II showed a much stronger shift than other risk factors. For cAI only the smoker group showed a shift to higher score results (predominantly for calculation rule 2 and 4).

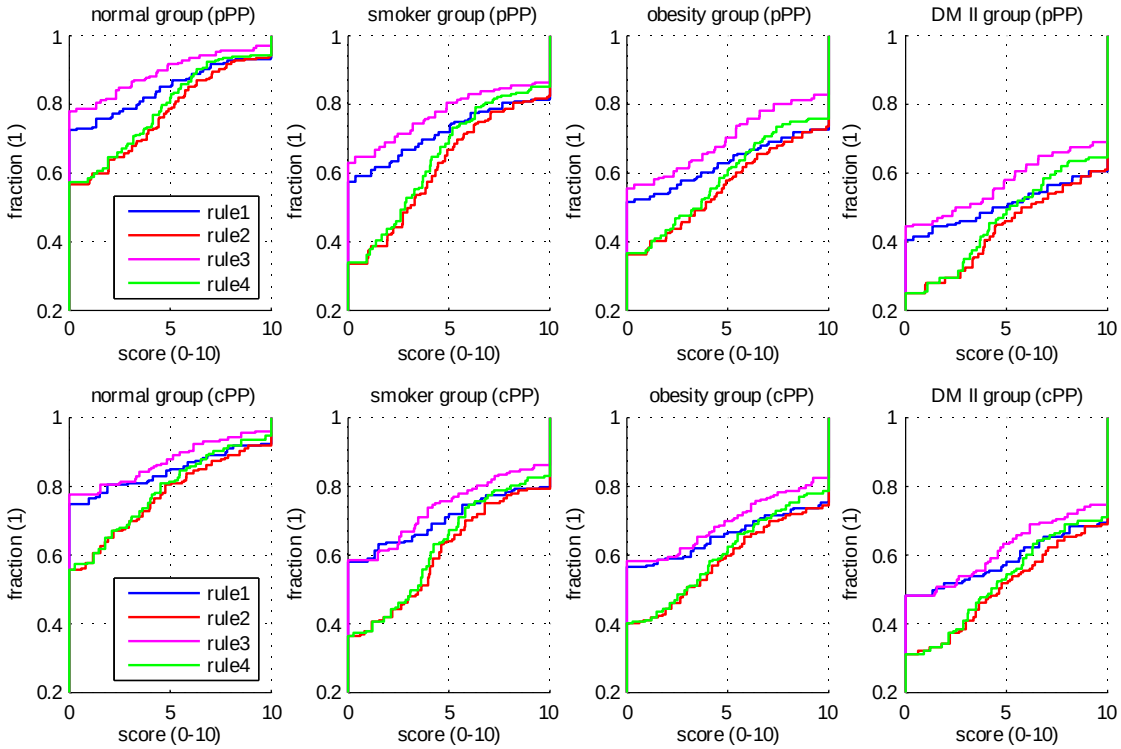


Figure 91: Comparison of reference value calculation rules for pPP and cPP. Plots are separated for pPP and cPP. Plots are also separated for the reference group and for each risk group. Results of each reference value calculation rule are indicated by different colors.

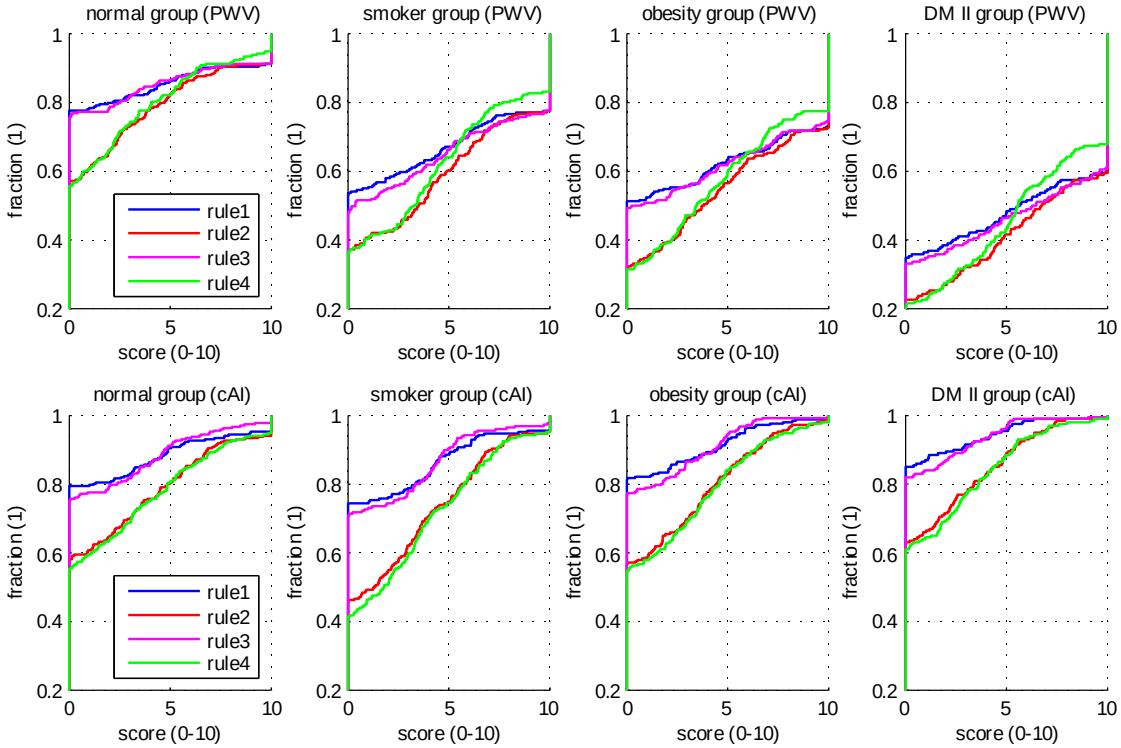


Figure 92: Comparison of reference value calculation rules for PWV and cAI. Plots are separated for PWV and cAI. Plots are also separated for the reference group and for each risk group. Results of each reference value calculation rule are indicated by different colors.

4.3.1.1. Risk groups compared with reference group

Figure 93 shows the calculated fractional differences between each risk group and the reference group calculated for calculation rule 1-4. For each risk group and reference value calculation rule a separate subplot is shown.

Following settings were used for this investigation: ***Group data:*** *male + female subjects;* ***reference values:*** *unisex reference values, calculated by rule 1, calculated by rule 2, calculated by rule 3, calculated by rule 4;* ***confounder correction:*** *no, uncorrected reference values for cAI and PWV.*

First of all, this investigation shows predominantly positive fractional differences but there were also negative fractional differences. Negative fractional differences are due to lower score results for the investigated subgroup compared to the associated reference subgroup.

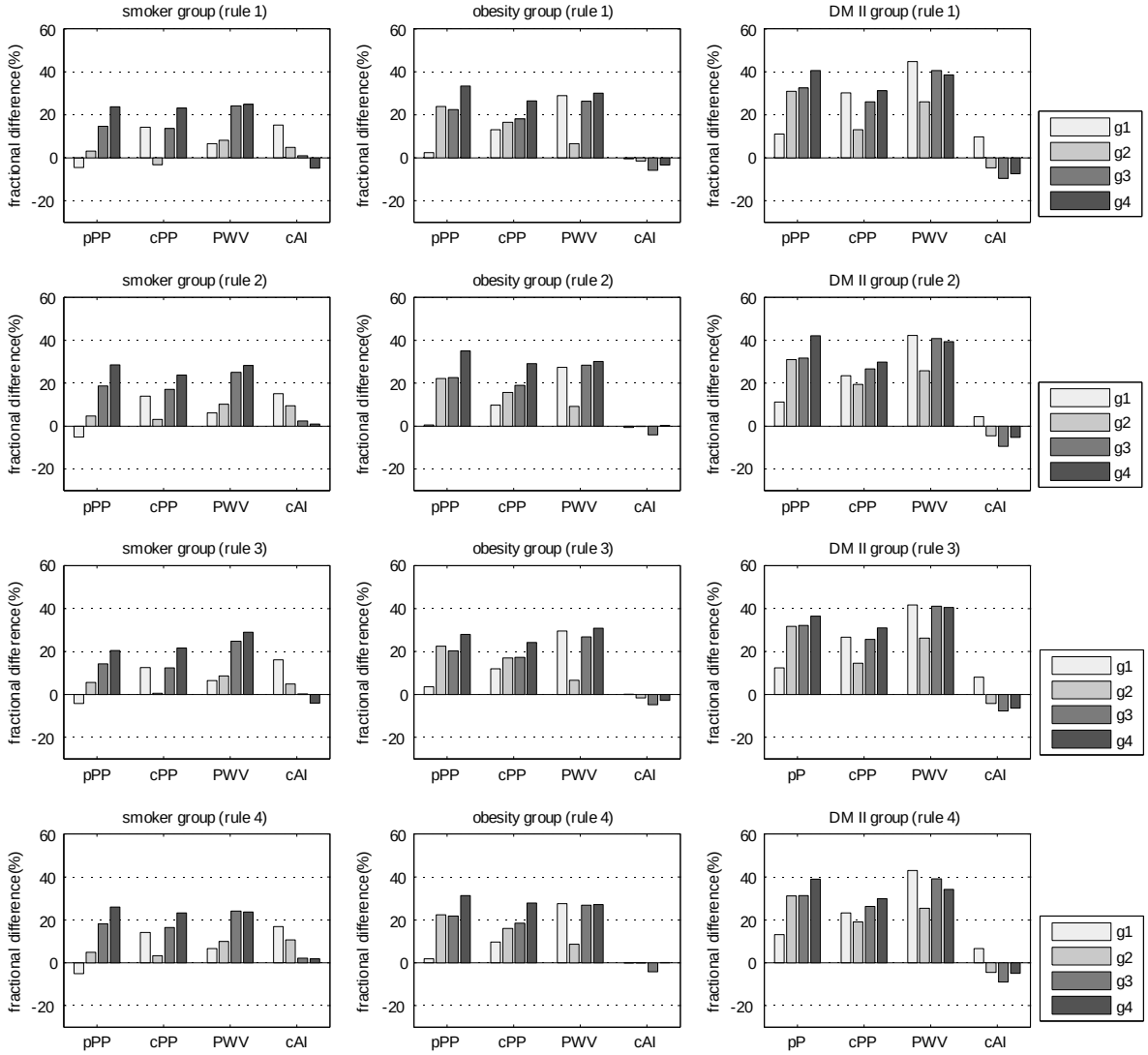


Figure 93: Comparison of fractional differences for risk groups calculated by each calculation rule. Plots are separated for each risk group and calculation rule. Each subplot shows fractional differences separated for each variable and age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

Comparison of reference value calculation rules: Fractional differences were nearly unchanged between calculation rules as shown in Figure 93.

Impact of risk factors: In Figure 93 it is shown that the impact of a risk factor on score results is dependent on age. For example, pPP shows an increase of fractional differences with age for each risk group. A similar result was found for cPP. Interestingly, cPP showed a notable decrease of fractional differences in the smoker and DM II group for age group 2. PWV showed an increase of fractional differences with age for the smoker group. The fractional differences of PWV were nearly unchanged for the obesity and DM II group but fractional differences of PWV showed a notable decrease for age group 2. The variable cAI showed an decrease of fractional differences with age for the smoker and the DM II group. For the obesity group fractional differences of cAI showed predominantly negative results. However, the DM II group showed the highest fractional differences of all three risk groups.

4.3.2. Impact of gender and confounder-correction

Reference values calculated based on the reference group showed some uncertainties for some gender-specific subgroups (see Table 3). However, reference values calculated by reference value calculation rule 2 showed the best results for subgroups with a low number of subjects. Due to this fact the impact of gender and the confounder correction on score results were investigated predominantly for calculation rule 2. Hence, this section presents results based on calculation rule 2. Furthermore, this section compares results calculated based on unisex reference values and regression lines with results calculated by gender-separated reference values and regression lines.

4.3.2.1. Impact of gender

It is important to note that gender-specific results had significant uncertainties for some age-specific subgroups due to a low number of subjects.

4.3.2.1.1. Results calculated by unisex reference values

Figure 94 shows the calculated fractional differences between each risk group and the reference group calculated based on unisex reference values. Results are separated for male and female subjects. *Following settings were used for this investigation:* ***Group data:*** *male subjects (top graphic); female subjects (bottom graphic);* ***reference values:*** *unisex reference values, calculated by rule 2;* ***confounder correction:*** *no, uncorrected reference values for cAI and PWV.*

Gender differences: Main differences between male and female subjects were found predominantly for the smoker and the obesity group: Male subjects showed a stronger increase of fractional differences for pPP, cPP and PWV with age whereas female subjects showed a tendency for a steady state or a slight increase and respective decrease with age. Additionally, male subjects showed higher fractional differences for older subjects (age group 3 and 4) for the variables pPP, cPP and PWV compared to female subjects. However, female subjects showed negative fractional differences for age group 4 in the smoker and obesity group. Furthermore, it is important to note that male subjects in age group 2

showed negative fractional differences for some risk groups and variables although there was an increase of fractional differences with increasing age.

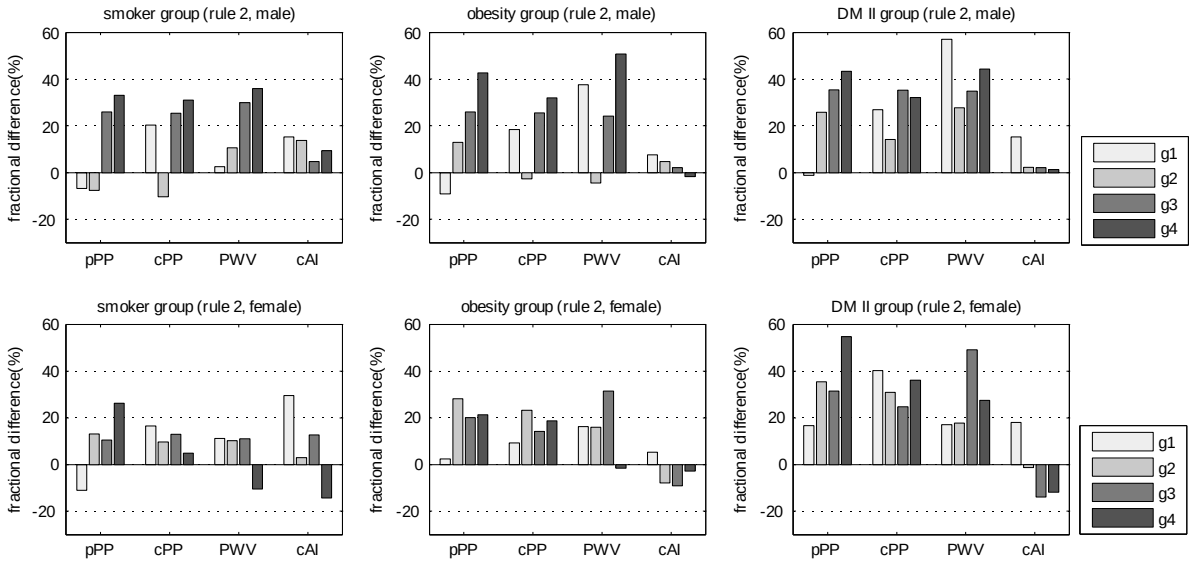


Figure 94: Comparison of fractional differences separated for male and female subjects (unisex reference values). Plots are separated for each risk group and gender category. Each subplot shows fractional differences separated for each variable and age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

4.3.2.1.2. Results calculated by gender-separated reference values

Figure 95 shows the calculated fractional differences between each risk group and the reference group calculated based on gender-separated reference values. Results are separated for male and female subjects. *Following settings were used for this investigation:* ***Group data:*** *male subjects (top graphic); female subjects (bottom graphic);* ***reference values:*** *separated reference values for male and female subjects, calculated by rule 2;* ***confounder correction:*** *no, uncorrected reference values for cAI and PWV.*

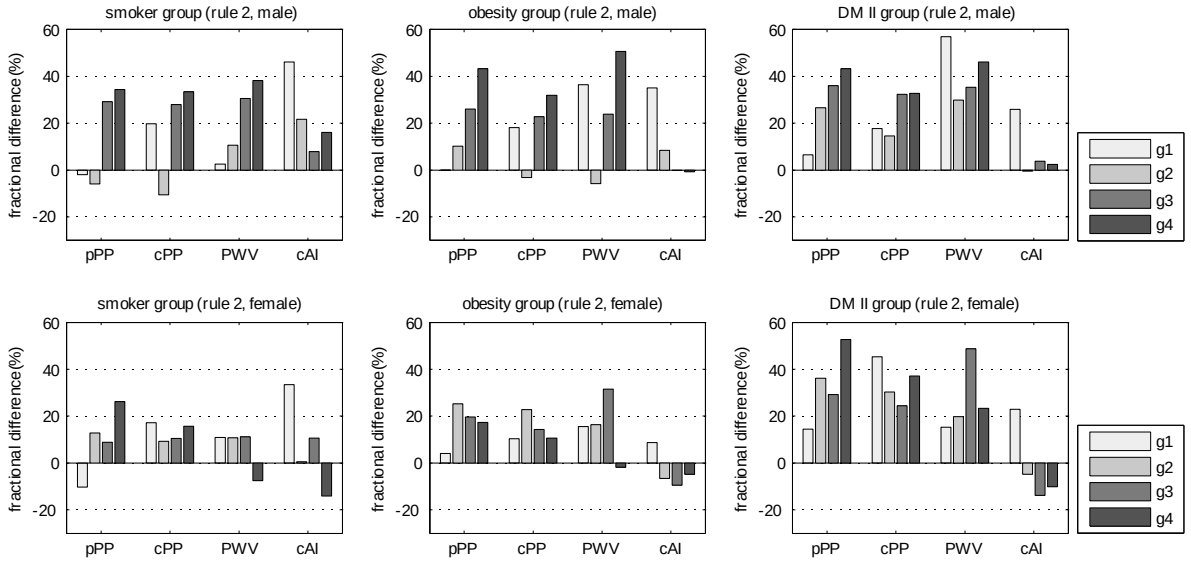


Figure 95: Comparison of fractional differences separated for male and female subjects (gender-separated reference values). Plots are separated for each risk group and gender category. Each subplot shows fractional differences separated for each variable and age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

Comparison with results based on unisex reference values: The use of gender-separated reference values showed only a slight impact on calculated fractional differences compared to results calculated by unisex reference values. However, a notable impact was found for the variable cAI in the obesity group for male subjects.

4.3.2.2. Impact of confounder correction

4.3.2.2.1. Results calculated by unisex reference values and regression lines

Figure 96 shows the calculated fractional differences between each risk group and the reference group calculated based on unisex reference values and regression lines. The top graphic shows results without a confounder-correction and the bottom graphic shows results with a confounder-correction. Confounder-corrected reference values were used additional to the confounder correction.

Following settings were used for this investigation: ***Group data:*** *male + female subjects;* ***reference values:*** *unisex reference values, calculated by rule 2;* ***confounder correction:*** *top graphic: no, uncorrected reference values for cAI and PWV; bottom graphic: for PWV and cAI, unisex regression lines, confounder-corrected reference values for cAI and PWV.*

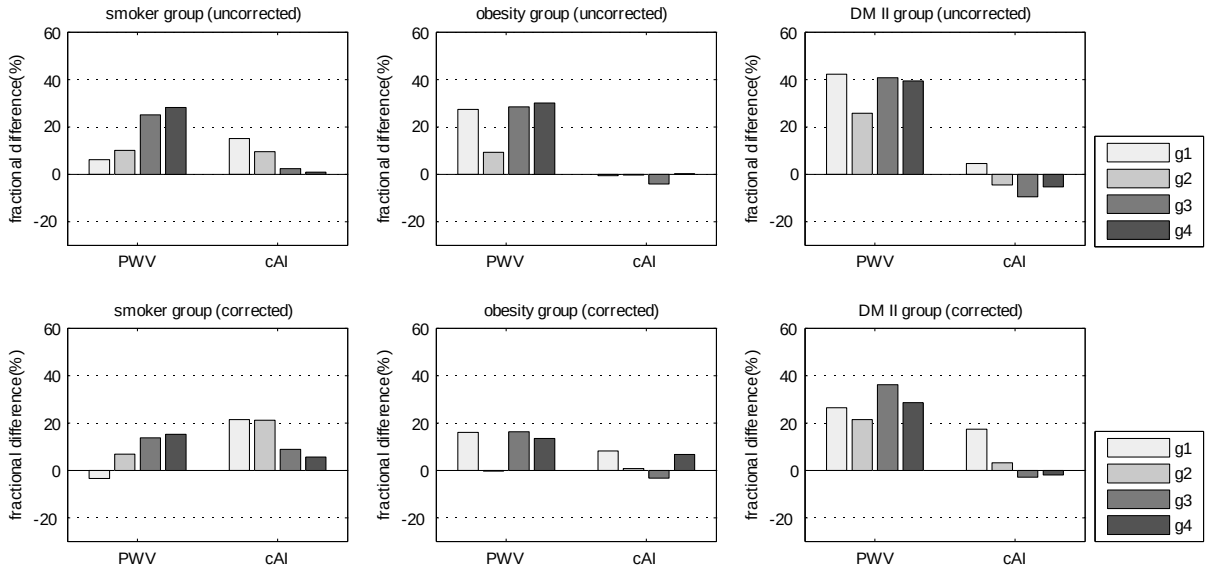


Figure 96: Impact of confounder-correction on score results (unisex reference values and regression lines). The top graphic shows results without a confounder-correction. The bottom graphic shows results with a confounder-correction. Plots are separated for each risk group. Each subplot shows fractional differences separated for each variable and age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

Uncorrected results versus confounder-corrected results: The confounder-correction led to a decrease of fractional differences for PWV. Same results were found for each risk group. In contrast, fractional differences for cAI increased slightly due to the confounder-correction.

4.3.2.2.2. Results calculated by gender-separated reference values and regression lines

Figure 97 shows the calculated fractional differences between each risk group and the reference group calculated based on gender-separated reference values and regression lines. The top graphic shows results without a confounder-correction and the bottom graphic shows results with a confounder-correction.

Following settings were used for this investigation: ***Group data:*** *male + female subjects;* ***reference values:*** *separated reference values for male and female subjects, calculated by rule 2;* ***confounder correction:*** *top graphic: no, uncorrected reference values for cAI and PWV; bottom graphic: for PWV and cAI, separated regression lines for male and female subjects, confounder-corrected reference values for cAI and PWV.*

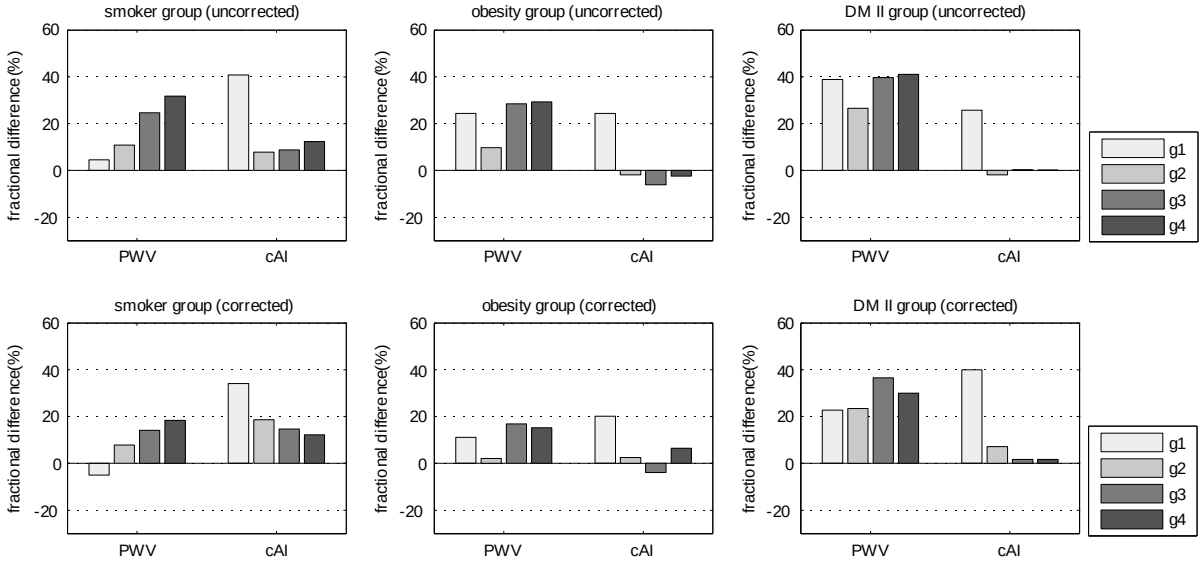


Figure 97: Impact of confounder-correction on score results (gender-separated reference values and regression lines). The top graphic shows results without a confounder-correction. The bottom graphic shows results with a confounder-correction. Plots are separated for each risk group. Each subplot shows fractional differences separated for each variable and age group (g1: 40-49 years, g2: 50-59 years, g3: 60-69 years, g4: 70-79 years).

Comparison with results based on unisex reference values and regression lines: The main difference was that fractional differences were higher for cAI (age group 1) in all three risk groups compared to results calculated based on unisex reference values and regression lines.

4.3.2.3. Comparison of results

The impact of different settings for reference values and regression lines (unisex or gender-sperated), and the impact of the confounder correction was compared. The resulted score data was plotted for: uncorrected results calculated by unisex reference values and regression lines, confounder-corrected results calculated by unisex reference values and regression lines, uncorrected results calculated by gender-separated reference values and regression lines, and confounder-corrected results calculated by gender-separated reference values and regression lines. Data were plotted as cumulative distribution.

Following settings were used for this investigation: ***Group data:*** *male + female subjects;* ***reference values:*** *unisex reference values (blue and magenta lines), separated reference values for male and female subjects (red and green lines), calculated by rule 2;* ***confounder correction:*** *no, uncorrected reference values for cAI and PWV (blue and red lines); for PWV and cAI, unisex regression lines for male and female subjects, confounder-corrected reference values for cAI and PWV (magenta lines); for PWV and cAI, separated regression lines for male and female subjects, confounder-corrected reference values for cAI and PWV (green lines).*

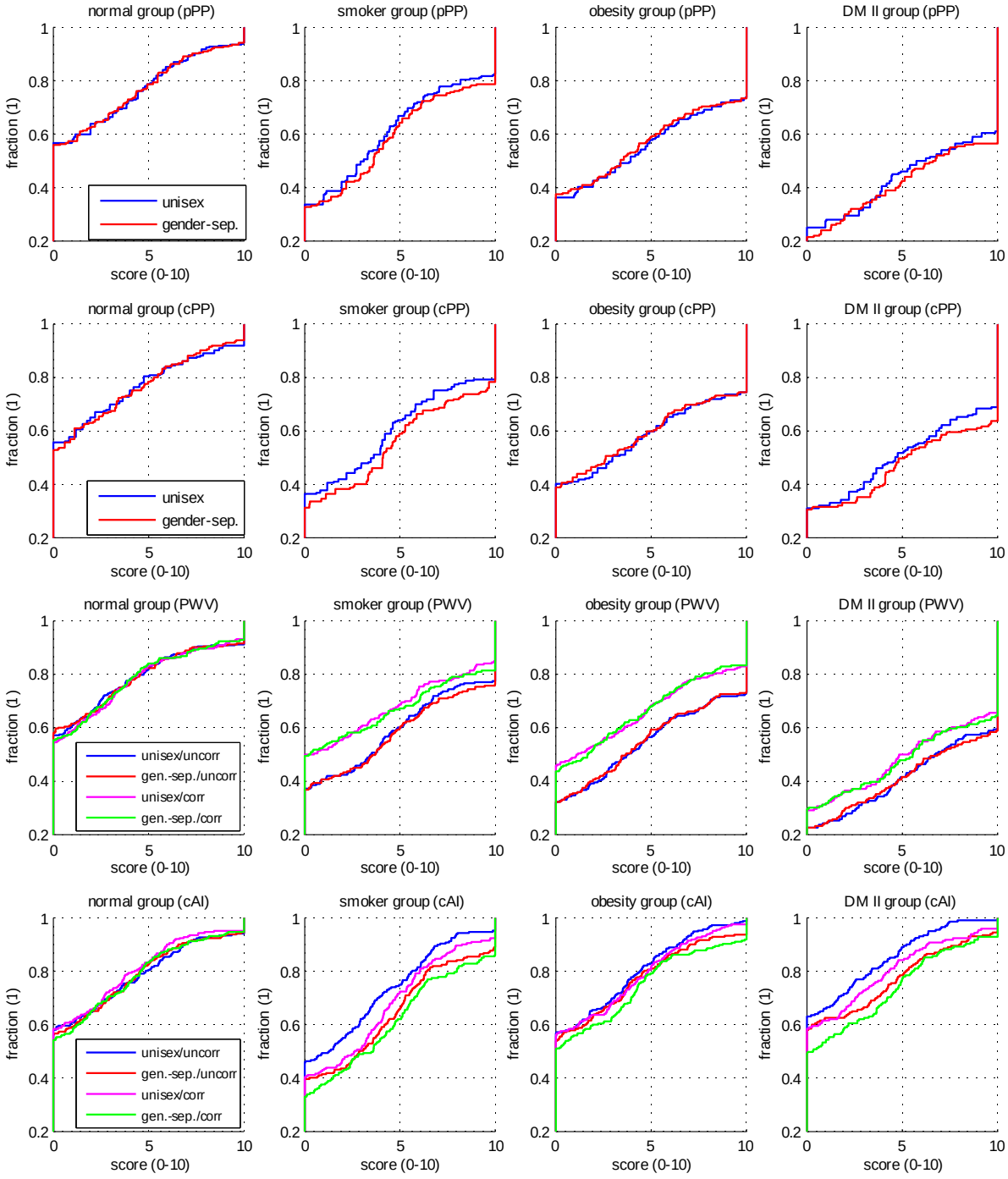


Figure 98: Comparison of results. Plots are separated for pPP, cPP, PWV and cAI. Plots are separated for the reference group and for each risk group. Blue lines: uncorrected results calculated by unisex reference values and regression lines; red lines: uncorrected results calculated by gender-separated reference values and regression lines; magenta lines: confounder-corrected results calculated by unisex reference values and regression lines; green lines: confounder-corrected results calculated by gender-separated reference values and regression lines.

pPP and cPP: For the smoker group and the DM II group a shift to higher score results was found for pPP and cPP due to the use of gender-separated reference values and regression lines.

PWV: There were only slight differences in score results due to unisex or gender-specific reference values and regression lines. However, the confounder-correction led to lower score results for each risk group. For the reference group, there were only slight differences due to the confounder-correction.

cAI: The use of gender-separated reference values and regression lines led to higher score results compared to unisex reference values and regression lines. This was predominately shown for the smoker group and the DM II group. The confounder-correction led also to higher score results. However, the differences between different settings of the confounder-correction, reference values and regression lines was much lower for the obesity group compared to the smoker and DM II group.

4.4. Discussion

Results indicate that surrogate variables of arterial stiffness and associated score results calculated based on surrogate variables are notably affected by risk factor exposure. Furthermore, results indicate that the impact on surrogate variables differs between age groups and therefore it can be hypothesized that the performance of surrogate variables as classification variables varies with age. However, it is important to note that for the investigation of the impact of risk factors on score results, the duration of the impact and the degree of the present risk factor (e.g. cigarettes per day) were not taken into account.

4.4.1. Characteristics of extracted risk groups

Table 4: Mean and standard deviation of blood indicators and blood pressure calculated for each risk group. This table summarizes the mean and the standard deviation of each blood indicator (TC, LDL cholesterol, HDL cholesterol and TG) and blood pressure (SBP and DBP) calculated for each risk group.

age group		**TC** (mmol/l)	**LDL** (mmol/l)	**HDL** (mmol/l)	**TG** (mmol/l)	**SBP** (mmHg)	**DBP** (mmHg)
smoker group	mean	5.32	3.17	1.47	1.59	134	78
	SD	1.03	0.92	0.42	0.92	18	9
obesity group	mean	5.56	3.41	1.39	1.81	135	80
	SD	1.07	0.95	0.42	0.95	17	9
DM II group	mean	4.27	2.29	1.19	1.84	138	76
	SD	0.99	0.80	0.35	0.99	19	8

Three risk groups were extracted from the ACCT data. The smoker group included 248 subjects, the obesity group included 262 subjects and the DM II group included 212 subjects. For the extraction of risk groups the definition of adequate filter criteria is important. Each risk group was extracted so that it included subjects with the defined risk factor and excluded subjects with the other two risk factors. Additionally, subjects receiving any vasoactive medication were also excluded. It is important to note that for the DM II group this was not possible, otherwise the number of subjects would decrease notably. This is due to the fact that most subjects with DM II has to use vasoactive medication. A

further limitation was that the definition of further filter criteria for each risk group would lower the number of subjects markedly. This would lead to age-specific subgroups without any subjects. However, Table 4 summarizes the mean and standard deviation of TC, LDL cholesterol, HDL cholesterol, TG, SBP and DBP for the extracted risk groups to give information on further risk factors. This table shows that the mean value of each indicator and risk group showed normal or borderline normal values compared to the guidelines as shown in Table 1 and Table 2. Interestingly, the DM II group showed lower values for TC and LDL cholesterol compared to the smoker and obesity group. This could be due to the fact that subjects with diabetes mellitus are treated for these risk factors by medication. However, further risk groups could be extracted based on blood indicators (e.g. subjects with high LDL cholesterol levels) form the ACCT data to investigate the impact on score results.

4.4.1.1. Uncertainties for gender-specific subgroups

Table 5: Number of subjects per subgroup of each risk group. Results are categorized for age and gender. Subgroups with a number of subjects lower than 20 are marked red.

age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
smoker group					
pPP	M+F	n=33	n=52	n=69	n=81
	M	n=17	n=19	n=45	n=72
	F	n=16	n=33	n=24	n=9
cPP	M+F	n=27	n=46	n=66	n=78
	M	n=16	n=15	n=43	n=70
	F	n=11	n=31	n=23	n=8
PWV	M+F	n=34	n=52	n=67	n=78
	M	n=17	n=19	n=43	n=70
	F	n=17	n=33	n=24	n=8
cAI	M+F	n=29	n=49	n=68	n=81
	M	n=16	n=17	n=44	n=72
	F	n=13	n=32	n=24	n=9
obesity group					
pPP	M+F	n=45	n=60	n=98	n=53
	M	n=26	n=18	n=35	n=30
	F	n=19	n=42	n=63	n=23
cPP	M+F	n=44	n=54	n=93	n=48
	M	n=26	n=16	n=34	n=29
	F	n=18	n=38	n=59	n=19
PWV	M+F	n=44	n=58	n=94	n=52
	M	n=25	n=18	n=34	n=30
	F	n=19	n=40	n=60	n=22
cAI	M+F	n=45	n=57	n=97	n=53
	M	n=26	n=18	n=35	n=30
	F	n=19	n=39	n=62	n=23
diabetes mellitus II					
pPP	M+F	n=12	n=34	n=86	n=70
	M	n=8	n=23	n=71	n=57
	F	n=4	n=11	n=15	n=11
cPP	M+F	n=11	n=34	n=84	n=66
	M	n=8	n=23	n=68	n=53
	F	n=3	n=11	n=16	n=11
PWV	M+F	n=12	n=31	n=84	n=65
	M	n=8	n=22	n=68	n=52
	F	n=4	n=9	n=16	n=11
cAI	M+F	n=11	n=34	n=87	n=70
	M	n=8	n=23	n=70	n=57
	F	n=3	n=11	n=17	n=11

It is important to note that the number of subjects varies between surrogate variables due to the fact that not every dataset was complete. Furthermore, the separation of each risk group into age and gender-specific subgroups leads to a low number of subjects for some subgroups. Table 5 summarizes the number of subjects for each subgroup. Subgroups with a low number of subjects are marked red. This table shows that especially subgroups with young subjects (age group 1 and age group 2) consisted of a low number of subjects. Furthermore, for female subjects in the DM II group no subgroup had a number of subjects greater than 20. It is obvious that results for gender-specific subgroups with a low number of subjects have higher uncertainties than subgroups with a higher number of subjects.

4.4.2. Impact of reference value calculation rules

Fractional differences were calculated between each risk group and the reference group to estimate the impact of risk factors on score results. However, the investigation of different reference value calculation rules showed that the shift of score results (fractional differences) in relation to the reference group is almost independent of the used reference value calculation rule as shown in Figure 99 (right figure). Hence, the used calculation rule defines how many subjects have low and respectively high score result in a specific group as shown in Figure 99 (left figure) but the calculation rule has only a slight impact on the relative shift of score values in relation to score results of a reference group (right figure).

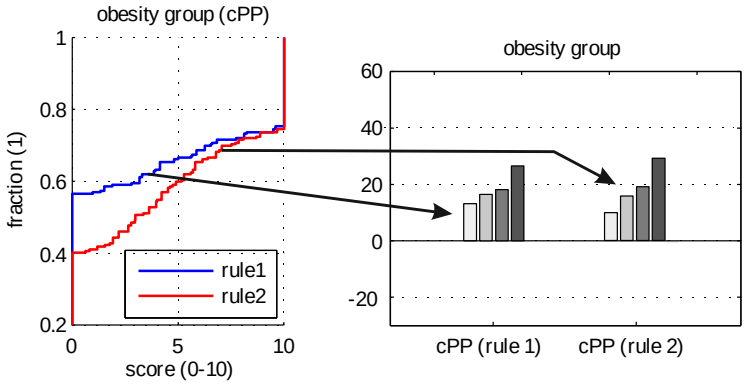


Figure 99: Impact of reference value calculation rules on the score distribution and fractional differences. This example shows that the score distribution of the obesity group is strongly affected by the reference value calculation rule (see left figure). This example also shows that fractional differences between the obesity group and the reference group differ only slightly (see right figure).

However, calculation rule 1 and 3 lead to lower number of subjects with a score of 0 compared to calculation rule 2 and 4. Thus, the definition of an adequate calculation rule is a key requirement for the calculation of reference values. Hence, a further investigation to define and validate a reference value calculation rule should be performed in a future investigation. Therefore, data of longitudinal cohort studies could be used as validation data.

4.4.3. Gender-separated reference values and regression lines

Differences between results calculated based on unisex reference values and regression lines, and results calculated based on gender-separated reference values and regression lines were only slight as

shown in Section 4.3.2. However, it is important to note that the use of gender-separated reference values had significant uncertainties due to a low number of subjects for some subgroups of the reference group (see Section 3.4.2.3). Hence, only results based on unisex reference values and regression lines are further discussed in the following sections. In addition, it is clear that gender-separated reference values and regression lines are important to account for gender-specific differences. A future investigation should use a reference group with a higher number of subjects to solve this important limitation.

4.4.4. Risk factors and score results

Results indicate that subjects under risk exposure show higher score results as subjects without risk exposure. The results can be further used to extract information on the sensitivity of the response of surrogate variables on risk factor exposure and on their different performance as classification variables between middle-aged (40-59 years) and elderly subjects (60-79 years).

However, results are discussed for middle-aged subjects (40-59 years) and elderly subjects (60-79 years) to reduce the impact of uncertainties due to a low number of subjects for some subgroups. Therefore, the mean of fractional differences for age group 1 and 2 (middle-aged subjects), and age group 3 and 4 (elderly subjects) is shown and discussed. Furthermore, calculation rule 2 is used for the discussion of the impact of risk factors on score results due to the fact that fractional differences were almost unchanged between reference value calculation rules.

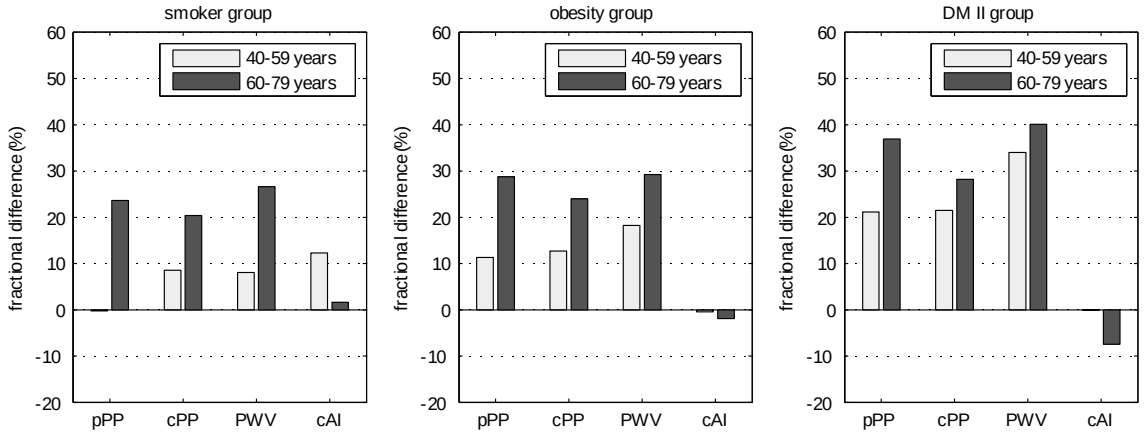


Figure 100: Impact of risk factors on score results. This figure shows the comparison of fractional differences for risk groups. Subplots are separated for each risk group. Each subplot shows fractional differences separated for each variable. Bars indicate middle-aged (40-59 years) and elderly subjects (60-79 years). Following settings were used for this figure: group data: male + female subjects; reference values: unisex reference values, calculated by rule 2; confounder correction: no, uncorrected reference values for cAI and PWV.

Impact of risk factors on score results: The data in Figure 100 indicate that DM II has the strongest impact on pPP, cPP and PWV and therefore on the score results of them. Hence, diabetes mellitus leads to a strong increase of arterial stiffness. These findings agree with findings from the literature. For instance, Schram et al. (2004) concluded that impaired glucose metabolism and DM II are associated with increased central artery stiffness, which is more pronounced in DM II, and deteriorating glucose tolerance is associated with increased central and peripheral arterial stiffness. In addition, PWV was markedly elevated in DM II subject. In contrast, cAI was nearly unchanged in the

DM II group. This result agrees with the findings of Lacy et al. (2004) and this study concluded that diabetes mellitus leads to an increase in PWV but this is not associated with an increased in cAI. Interestingly, the smoker group showed higher fractional differences for cAI than other risk groups. It was shown that smoking leads to an increase in total peripheral resistance (Omvik, 1996). Therefore it could be hypothesized that this leads to an increase in wave reflections and further to an increase in cAI. The obesity group showed similar results as the DM II group but on a lower level. However, there is still a lack of knowledge regarding the pathophysiological mechanisms of obesity in relation to arterial stiffness.

Score results and age: As shown in Figure 100 the impact of risk factors on score results differ between middle-aged and elderly subjects. Each risk group showed an increasing trend for pPP, cPP and PWV for each risk group but cAI showed an decreasing trend with age. Additionally, negative fractional differences were found predominately for pPP especially in younger subjects, and for cAI especially in older subjects meaning these groups had lower score results compared to the reference group. This could be due to the fact that cAI is an 'indirect measurement' of arterial stiffness, since cAI is also affected by wave propagation in addition to elasticity changes in the arterial wall (Nichols and O'Rourke, 1998, p201-p222). Some investigations found a plateau effect after the age of 55 or 60 years (McEniery et al. (2005) and Fantin et al. (2007)). The same situation was found in the ACCT data (see Figure 36). As a result cAI was shown to be a good surrogate variable of arterial stiffness in middle-aged subjects (40-59 years) and a weak surrogate variable in elderly subjects (60-79 years). This indicates that cAI cannot be used as classification variable for arterial stiffness over the age of 60, possibly because of changes in the reflection mechanisms. Furthermore, the resulting negative fractional difference for pPP in the smoker group indicates that pPP has a reduced usefulness as classification variable for middle-aged smokers. However, it is important to note that no information was obtained on how long subjects have had the risk factor at the time of measurement. Hence, for a more detailed investigation this information is important.

Conclusion: In conclusion, the comparison of risk group results indicates that DM II has a very strong impact on the structure of the arterial wall and leads to a notable decrease of arterial elasticity. Smoking is a stronger determinant of changes in the wave reflection mechanisms and pPP should not used as classification variable in young smokers. The variable cAI is not useful as indicator for elderly subjects especially in subjects with obesity or DM II. Furthermore, cPP and PWV can be used as indicator over the whole investigated age range.

4.4.5. Role of gender and confounder-correction

4.4.5.1. Impact of gender

The findings of this analysis showed that gender-separated results had significant uncertainties due to the low number of subjects in some subgroups of the risk groups (see Section 4.4.1.1). Furthermore, gender-separated results differed only slightly between the calculation with unisex reference values and the calculation with gender-separated reference values (see Figure 94 and Figure 95). However, separated results for male and female subjects showed a large impact on score results predominantly

for female subjects. Figure 101 shows the fractional differences for middle-aged and elderly subjects separated for gender. This figure shows that male subjects had similar results as calculated by unisex data but female subjects showed a fundamental different behavior for smokers and obese subjects.

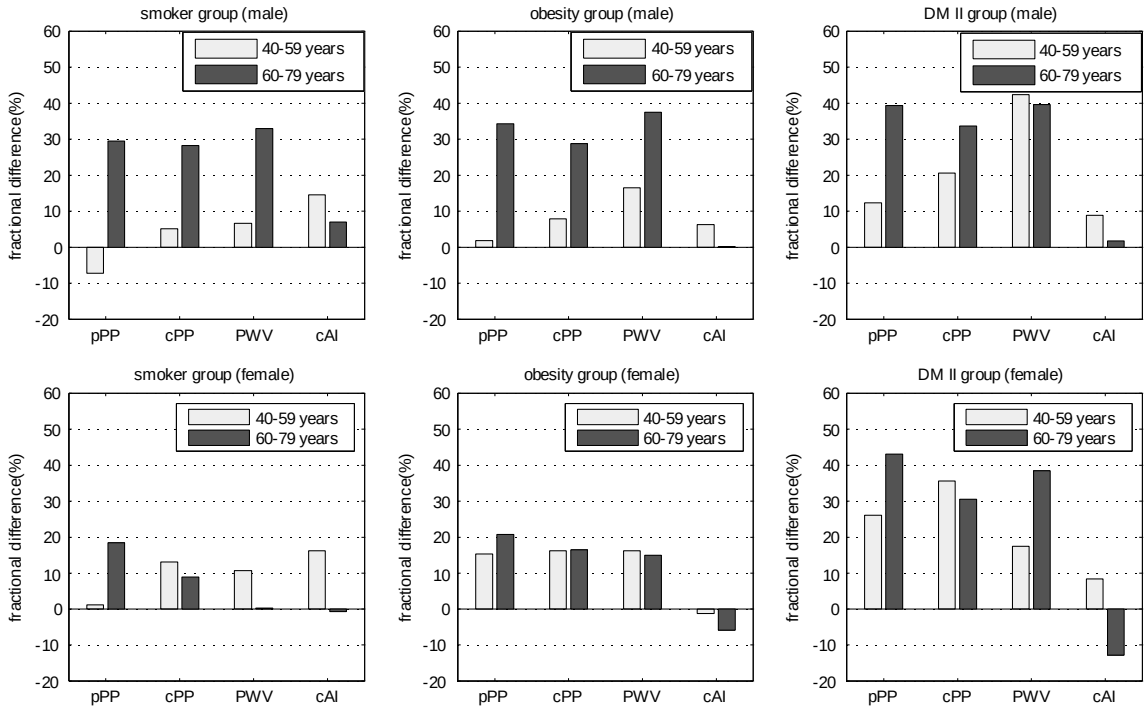


Figure 101: Impact of risk factors on score results separated for gender. This figure shows the comparison of fractional differences for risk groups. Subplots are separated for each risk group and gender. Each subplot shows fractional differences separated for each variable. Bars indicate middle-aged (40-59 years) and elderly subjects (60-79 years). Following settings were used for this figure: group data: male subjects (top graphics), female subjects (bottom graphics); reference values: unisex reference values, calculated by rule 2; confounder correction: no, uncorrected reference values for cAI and PWV.

Female smokers and female obese subjects: In contrast to male subjects, female subjects showed a decrease of fractional differences for cPP and PWV with age. Furthermore, cPP and PWV were nearly unchanged in the obesity group. That is, results of cPP and PWV differed mainly between male and female subjects. However, for a further interpretation of these results additional investigations with more subjects should be performed.

Male subjects in age group 2: There was a notable decrease of fractional differences for male subjects (age group 2) in the smoker and obesity group as shown in Figure 94. This could be a further result of a low number of subjects as shown in Table 5.

4.4.5.2. Impact of the confounder-correction

It is important to note that the confounder-correction was limited due to the fact that regression equations were calculated based on inter-individual data (see Section 3.4.4.1). However, the comparison of confounder corrected results (see Figure 102) with uncorrected results (see Figure 100) for PWV and cAI showed the following characteristics: the confounder-correction of PWV for the impact of MAP led to a notable decrease of fractional differences for each risk group. However, each risk group showed higher PWV scores compared to the reference group. Therefore, it could be

hypothesized that a part of the increase of PWV is due to a structural change of the arterial wall and another part of the increase is due to an increased of the MAP level in risk subject. Furthermore, the confounder-correction of cAI for the impact of HR and body height showed an increase of fractional differences predominantly for DM II subjects. This could be due to the fact that the DM II group consisted of more male subjects (158 subjects) than female subjects (42 subjects). Male subjects are statistically taller than female subjects. Therefore the impact of the confounder-correction on results was stronger compared to other risk groups.

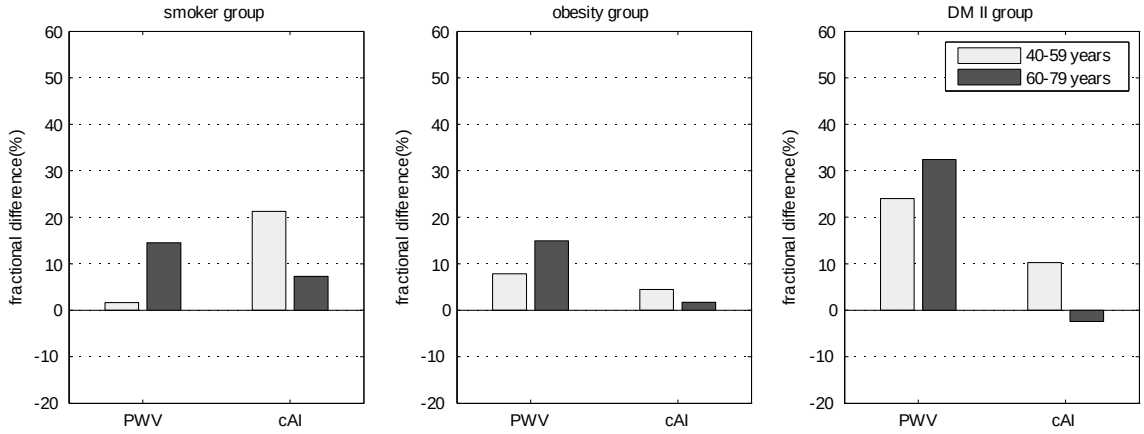


Figure 102: Impact of confounder-correction on score results. This figure shows the comparison of fractional differences for risk groups. The confounder-correction was used for PWV and cAI. Subplots are separated for each risk group. Each subplot shows fractional differences separated for each variable. Bars indicate middle-aged (40-59 years) and elderly subjects (60-79 years). Following settings were used for this figure: group data: male + female subjects; reference values: unisex reference values, calculated by rule 2; confounder correction: for PWV and cAI, unisex regression lines, confounder-corrected reference values for cAI and PWV.

4.5. Conclusions

The present investigation shows that score results calculated by the FLSS based on surrogate variables of arterial stiffness can be used to assess the impact of risk factor exposure on score results. Furthermore, it was shown that the performance of surrogate variables of arterial stiffness as classification variables varies with age.

Main findings

The present investigation shows that DM II has a very strong effect on the structure of the arterial vessel wall and leads to a notable decrease of arterial elasticity. Smoking is a stronger determinant of changes in wave reflection mechanisms and pPP should not be used as classification variable in young smokers. The variable cAI is not useful as indicator for elderly subjects especially in subjects with obesity or DM II. Furthermore, cPP and PWV can be used as indicator over the whole investigated age range.

Main limitations and future work

Some gender-specific results showed uncertainties due to the fact that some subgroups of each risk group had a low number of subjects. Hence, a further investigation with more subjects per subgroup should be performed. However, for the investigation of the impact of risk factors on score results, the duration of the impact and the degree of the present risk factor (e.g. cigarettes per day) were not taken into account. In addition, it will be important to investigate the impact of further risk factors such as high TC, high LDL cholesterol or salt intake. Furthermore it will be important to investigate the impact of vasoactive medication.

5. Discussion

5.1. Novel aspects of calculated scores based on arterial stiffness

Established risk scores such as CVD or CHD risk scores (e.g. risk scores based on the Framingham study) are based on the measurement of traditional risk factors such as blood indicators (e.g. TC, LDL cholesterol or HDL cholesterol), blood pressure (e.g. SBP) or lifestyle factors (e.g. smoking). Hence, these types of scoring systems are focused on the assessment of cardiovascular risk and they give statistical information of the probability of a subject having a cardiovascular event in a specific time-period (e.g. 10-years risk for having a heart attack).

In contrast, the present scoring concept and scoring technique based on surrogate variables of arterial stiffness give no information of the cardiovascular risk of a subject. Therefore, it was hypothesized (see Section 1.7.3.1) that surrogate variables of arterial stiffness can be used to give information on the structural change of arterial vessels expressed as pathophysiological change of arterial stiffness. However, it was shown (see Chapter 3 and 4) that surrogate variables of arterial stiffness increase with age and this change is accelerated for subjects exposed to risk factors such as smoking, obesity or DM II. Hence, the present score can be seen as a measure of the structural change of arterial vessels expressed as an increase of arterial stiffness due to risk factors or genetic predisposition in relation to age-specific normal values of surrogate variables. Furthermore, based on the concept of normal and early vascular aging (see Section 1.5.2) a pathophysiological increase of arterial stiffness and the resulting increase of scores can be further interpreted as early vascular aging.

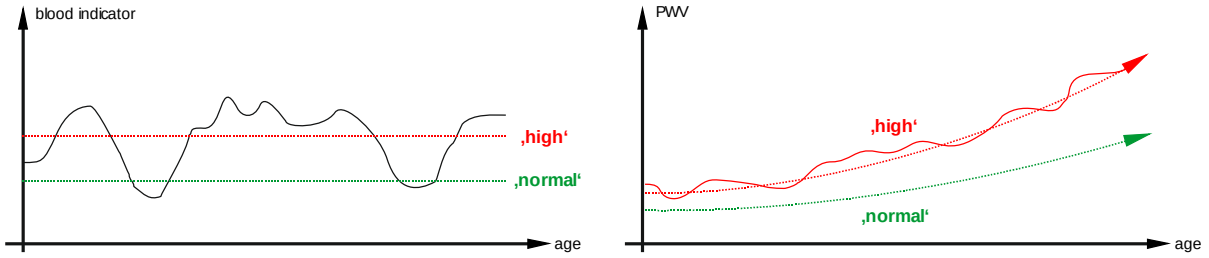


Figure 103: Blood indicator versus surrogate variable of arterial stiffness with age. This figure shows the difference between the information of a blood indicator (left figure) and the information of arterial stiffness (right figure).

There is an important difference between the informative content of traditional risk factors such as blood indicators (TC, LDL cholesterol or HDL cholesterol) and the informative content of surrogate variables of arterial stiffness. An increase of blood indicators gives information related to an increase of cardiovascular risk, but blood indicators can change over a short period of time (see Figure 103, left graphic). That suggests that for the lifetime of a subject a blood indicator can be high for some years and later can be low due to a change of lifestyle. In contrast, the present investigation (see Chapter 3 and 4) shows that surrogate variables give information on the structural change of the arterial vessels (accelerated increase in arterial stiffness) due to the impact of risk factors such as high TC or LDL cholesterol. In follows, that the increase of arterial stiffness is a cumulative process which is

accelerated for risk subjects (see Figure 103, right graphic). In conclusion, arterial stiffness can be used to assess the current status of the arterial vessels whereas blood indicators give information related to an increase in cardiovascular risk.

The following figure summarizes the discussed relationships: It is shown that a long-term impact of traditional risk factors such as smoking, DM II, high blood pressure or information on blood indicators leads to an accelerated increase in arterial stiffness with age. However, evidence shows that an increase in arterial stiffness is also a potential marker for an increase of cardiovascular risk (Vlachopoulos et al., 2010). Therefore it is clear that an increase of the score calculated by the FLSS, which reflects the increase of arterial stiffness, must have an additional relationship to an increase in cardiovascular risk. Hence, it is of significant importance to investigate this relationship in a future study based on longitudinal cohort data.

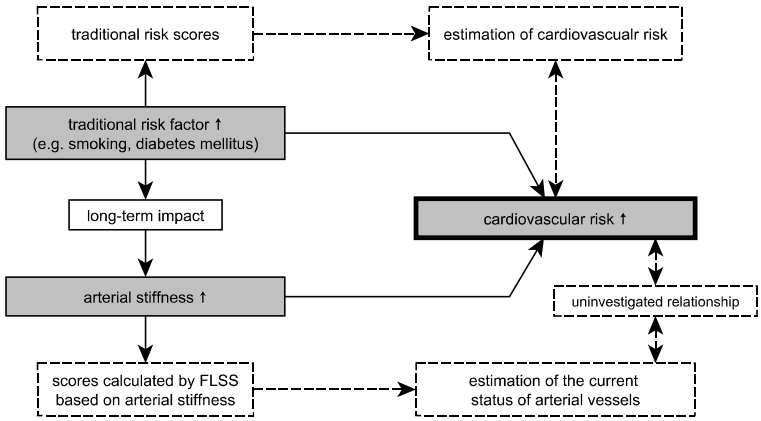


Figure 104: Traditional risk factors versus surrogate variables of arterial stiffness. This figure shows the difference between the information of traditional risk factors such as smoking or blood indicators and the information of arterial stiffness.

5.1.1. Conclusions of novel aspects

In conclusion, the present scoring system reflects structural changes of the arterial system measured as a pathophysiological increase of arterial stiffness due to the impact of risk factors or genetic predisposition and it gives no information related to the risk for having a future cardiovascular event. Hence, risk scores give longitudinal information for future events and the present score based on surrogate variables of arterial stiffness gives cross-sectional information on the normality or abnormality of variable at the time of measurement.

5.2. Additional value of the presented scoring technique

A main advantage of the scoring concept is that it takes physiological aging of the cardiovascular system into account and it differs between a physiological value and a pathophysiological value of an indicator or marker in relationship to the age of a subject. A further advantage of the model is that it can be extended easily for additional input variables if the necessary external data (reference values and regression equations) are available. Additionally, it takes the gender of a subject and the impact of

confounders of the measurement into account. Furthermore, calculated scores give abstracted information (score from 0 to 10) on the cardiovascular health status and therefore it can be easily interpreted by subjects. In conclusion, the present scoring technique and FLSS based on easily measurable surrogate variables of arterial stiffness can be used for screening procedures, preventive examinations or can be implemented into devices.

5.3. Different informative value of surrogate variables

Each surrogate variable of arterial stiffness reacts differently for an increase of arterial stiffness because each surrogate variable is influenced by different mechanisms. Therefore, surrogate variables of arterial stiffness cannot be used as interchangeable surrogate variables for arterial stiffness. These relationships were investigated in Chapter 4. This investigation shows that smoking has a fundamentally different effect on surrogate variables than DM II and obesity. Hence, it is important to note that score results have to be interpreted differently depending on the present risk factor. For instance, cAI plays an important role for middle-aged smokers whereas cAI is not important for the classification of diabetes mellitus or obese subjects. In addition, pPP should not be used as classification variable for middle-aged smokers. Furthermore, cPP and PWV can be used as indicator for each investigated risk factor over the whole investigated age range. In conclusion, this investigation showed that the usefulness of each surrogate variable as classification variable varies with age and between risk factors. Hence, it will be important for future guidelines to give information on which surrogate variables are more important as classification variables for different classes of age and present risk factors to improve screening procedures.

5.4. The usefulness of fuzzy logic for the development of the scoring system

The use of fuzzy logic has an important benefit for the implementation of the scoring concept due to the fact that fuzzy logic can be used to describe smooth transitions from normal to abnormal values and further from physiological to pathophysiological levels. Furthermore, fuzzy logic is readily understandable by users and can be easily extended for the implementation of further components such as confounder-correction.

5.5. Main limitations

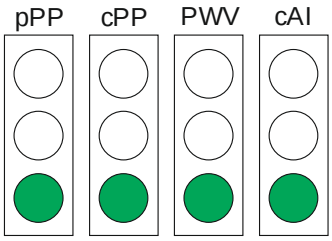
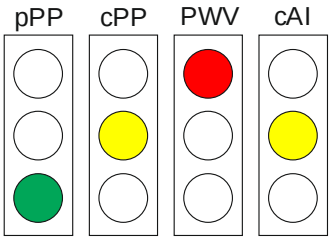
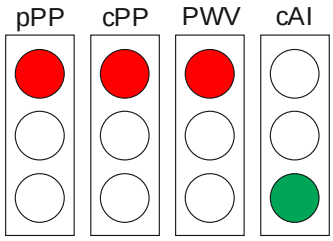
The present scoring technique and setup procedure for surrogate variables of arterial stiffness has a variety of limitations. Some limitations are due to a lack of data and some limitations are due to methodical limitations. It is important to note that the definition of reference values with reference value calculation rules were in a specific range arbitrary (see Section 3.4.3.1) and linear regression equations based on inter-individual data can only be seen as an rough approximation of relationships between input variables and confounders. Therefore, intra-individual data would be necessary. In addition, the definition of a linear increasing score is arbitrary as well and has to be investigated in a further study. However, gender-specific results had strong uncertainties due to a lack of gender-specific data. Therefore, additional data are needed as well. An additional limitation was that only subjects aged from 40 to 79 years were used for the setup procedure and for the investigation of risk

factors. Hence, the investigation of younger subjects could be used to extend the scoring system for younger subjects and for a further validation of the present results. However, for the investigation of the impact of risk factors on score results, the duration of the impact and the intensity of the present risk factor were not taken into account.

5.6. Practical examples

Examples are presented in the following tables to show the practical use of the FLSS based on surrogate variables of arterial stiffness. Table 6 shows three examples of subjects with either no or one risk factor (smoking or DM II).

Table 6: Practical examples for subjects with either no or one risk factor. Three different examples are shown. Example 1 shows a subject without an overt risk factor. Example 2 and 3 shows examples with one overt risk factor.

Example 1		**Example 2**		**Example 3**	
female, age=53 years		female, age=49 years		male, age=57 years	
no overt risk factor		smoker		diabetes mellitus II	
MAP=78mmHg HR=53bpm height=1.7m		MAP=98mmHg HR=56bpm height=1.67m		MAP=104mmHg HR=77bpm height=1.69m	
pPP=36mmHg	pPPscore=0	pPP=43mmHg	pPPscore=0	pPP=74mmHg	pPPscore=10
cPP=30mmHg	cPPscore=0	cPP=39mmHg	cPPscore=5.8	cPP=54mmHg	cPPscore=10
PWV=5.39m/s	PWVscore=0	PWV=8.51m/s	PWVscore=10	PWV=10.21m/s	PWVscore=10
cAI=29.3%	cAIscore=0	cAI=45.8%	cAIscore=9.5	cAI=21.0%	cAIscore=0
					

Results are indicated as traffic lights where a score of 0 leads to a ‘green’ traffic light, a score greater 0 and lower 10 leads to a ‘yellow’ traffic light and a score of 10 leads to a ‘red’ traffic light. Calculated scores for PWV were corrected for MAP and calculated scores for cAI were corrected for HR and body height. Example 1 shows a middle-aged female subject with no overt risk factor. This woman had low values for pPP, cPP, PWV and cAI. Hence, each score was 0 and each traffic light (‘green’) indicates that each measured variable was normal. Example 2 shows a female smoker. For this subject cPP and cAI were elevated (traffic light ‘yellow’) and PWV was high (traffic light ‘red’). However, pPP was ‘normal’. In Chapter 4 it was shown that for middle-aged smokers cPP, PWV and cAI are predominately affected by this risk factor. In contrast, example 3 (male subject with DM II) shows that diabetes mellitus affects predominantly pPP, cPP and PWV.

Table 7 presents four examples based on the measurement of PWV to show that the FLSS accounts for the effect of age. This table shows that for each subject in each example the score result was

approximately 5 although example 3 and 4 had a much higher PWV value compared to example 1 and 2. This is due to the fact that the FLSS accounts for age and therefore with increasing age a higher PWV value is more acceptable.

Table 7: Practical examples to show the impact of age on score results. This table shows four examples with subjects of different age. Each example had a PWV score result of approximately five.

Example 1	Example 2	Example 3	Example 4
female, age=45 years	male, age=56 years	female, age=65 years	male, age=74 years
MAP=88mmHg PWV=7.10m/s	MAP=89mmHg PWV=8,29m/s	MAP=89mmHg PWV=9.03m/s	MAP=88mmHg PWV=10.42m/s
PWVscore=5.2	PWVscore=4.9	PWVscore=5.1	PWVscore=5.4

In addition, this example shows that a threshold value of 12 m/s as suggested by Mancia et al. (2007) (see Figure 79) cannot be sufficient for each age category due to the fact that it is physiological for older subjects to have an higher PWV value compared to younger subjects. Hence, a threshold value would classify a lot of young subjects as healthy subjects and a lot of old subjects as diseased subjects. Therefore, the present scoring system accounts for physiological aging.

5.7. Open questions and future research

This work shows that the scoring concept and scoring technique based on surrogate variables of arterial stiffness can be used for the assessment of the structural change of arterial vessels expressed as pathophysiological increase of arterial stiffness in relation to normal values. However, for the use of the scoring technique in clinical practice and further for devices, a variety of investigations based on additional amount of data and further validation steps are required. Hence, the present work can be interpreted as a first step to establish the novel scoring technique.

The following section gives information on the most important future work to validate and establish the scoring technique:

Additional input variables: Currently, the scoring system has been setup for pPP, cPP, PWV and cAI. Additional markers or indicators of the arterial health status such as distensibility coefficients, intima media thickness or endothelial function could be added to the model. However, adequate data for the calculation of reference values, regression equations and weighting factors are needed for a further extension of the scoring system.

Additional age groups: The present scoring system was setup for two age groups for middle-aged subjects (g1: 40-49 years, g2: 50-59 years) and two age groups for elderly subjects (g3: 60-69 years, g4: 70-79 years). The definition of further age groups for younger and older subjects would have an additional value for the practical use of the scoring system.

Gender-specific results: To generate more evidence of gender-specific results, it will be necessary to use additional datasets which have more subjects per subgroup.

Duration of the impact and the intensity of a present risk factor: It will be important to investigate the different impact of a risk factor which was present for either a long or short time period for a subject. In addition, the impact due to the degree of a risk factor such as the number of cigarettes per day for smokers should be investigated.

Investigation of score results for single subjects: It was shown that the scoring system can be used for the classification of group results. However, it will be important to investigate the correctness of the score calculation for single subjects.

Impact of further confounders: In addition, it is important to standardize the measurement procedures. For instance, all measurements should be performed in supine position. Furthermore, medication can influence the measurement of the presented variables (pPP, cPP, PWV and cAI). Therefore, the impact of vasoactive medication should be investigated and measurement procedures should be standardized.

Relationship of the present score with cardiovascular risk: As described in Section 5.1, the investigation of the relationship of an increase of the present score with an increase of cardiovascular risk is important. Therefore, data of a prospective cohort study would be necessary.

5.7.1. Reproducibility and measurement errors of measurements

The investigation of measurement errors and reproducibility of results of the different measurement procedures and devices to assess surrogate variables of arterial stiffness is still an important research question. However, the impact of measurement errors has an important effect on the calculated score results. The measurement errors vary between measurement techniques, devices and algorithms. For instance, for the assessment of PWV a variety of techniques and algorithms are used. For example, Millasseau et al. (2005) showed that different algorithms can lead to differences in calculated PWV values of 5 to 15 %. If a linear increasing score is assumed this would lead to a change in PWV score results of approximately 2 to 5 score points. This shows the importance of device standardization, algorithms and measurement procedures for the calculation of adequate score results. In addition, the measurement of the travel distance (Δx) (see (35)) for PWV or the use of different calculation algorithms (transfer functions (see Section 3.1.1.3.1)) for the calculation of the central aortic pressure waveform has additional uncertainties and should be investigated. However, these examples show the importance for these future investigations to establish the present scoring system based on evidence.

5.7.2. Overall scores and additional risk factors

The use of single output scores for each surrogate variable of arterial stiffness (pPP score, cPP score, PWV score and cAI score) will not be sufficient for the practical use of the scoring system. Therefore, it will be important to connect single output scores by using weighting factors to an overall output as shown in Figure 105. Details about the calculation of overall scores by the FLSS can be found in Chapter 2. Hence, the procedure of the calculation of adequate weighting factors for the calculation of

an overall score based on single output scores of surrogate variables will be an important future task for the establishment of the FLSS.

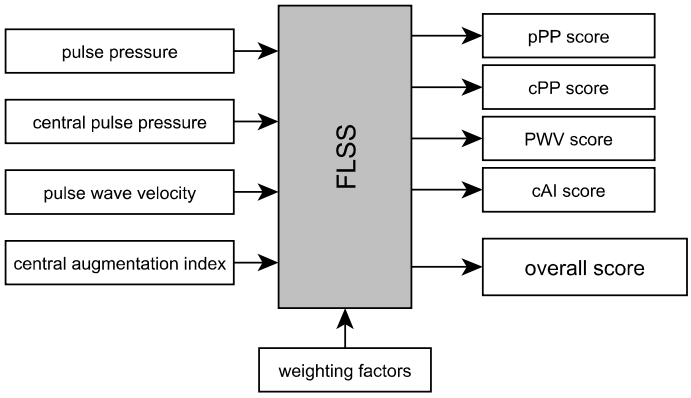


Figure 105: Calculation of an overall score based on weighted single output scores. The overall score is calculated based on the weighted single output scores for each used input variable.

Results of investigated risk factors (smoking, obesity and DM II) (see Chapter 4) indicate that a different set of weighting factors should be defined for each investigated risk factor. The following figure shows estimated weighting factors based on the results of Chapter 4.

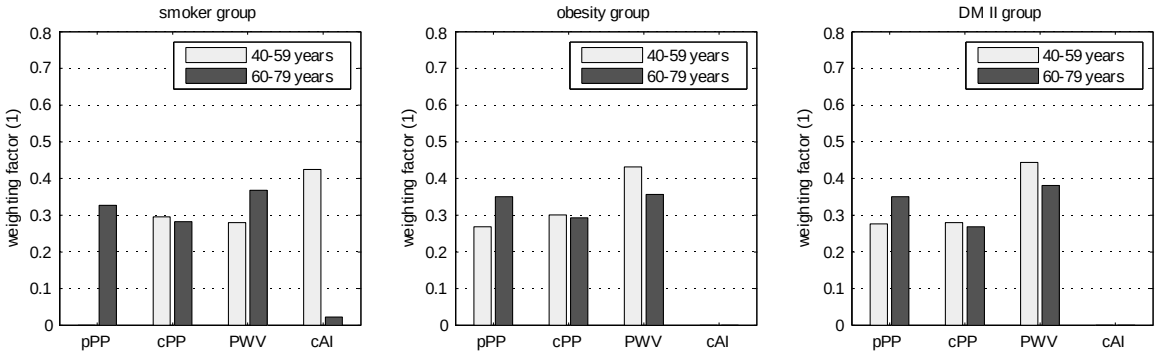


Figure 106: Weighting factors for overall score. This figure shows the comparison of weighting factors for risk groups. Subplots are separated for each risk group. Each subplot shows weighting factors separated for each variable. Bars indicate middle-aged (40-59 years) and elderly subjects (60-79 years). Following settings were used for this figure: group data: male + female subjects; reference values: unisex reference values, calculated by rule 2; confounder correction: no, uncorrected reference values for cAI and PWV.

This example shows that the set of weighting factors would be similar for obese subjects and for subjects with DM II. This example shows also that cAI does not play any role as classification variable for obese subjects and subjects with DM II. In contrast, for smokers cAI would be more important for middle-aged subjects and pPP would be more important for elderly subjects. Hence, this example shows that weighting factors vary between risk groups and therefore adequate weighting factors have to be investigated in a future study. In addition, it would be also important to estimate the effect on subjects with more than one risk factor and it would be important to estimate the impact of other risk factors such as salt intake, high TC or high LDL cholesterol. Furthermore, it could have an additional benefit to use information of prospective cohort studies for the estimation of weighting factors.

6. Conclusions

This work presents a scoring concept and a scoring technique based on fuzzy logic to assess the cardiovascular health status of a subject. An important novel aspect of the developed scoring system is that it takes the physiological aging of the cardiovascular system into account and it differs between a physiological value and a pathophysiological value of an indicator or marker in relation to the age of a subject. In addition, the scoring system accounts for gender and the impact of confounders of the measurement. Additionally, the present scoring system can be extended easily and used for different indicators or markers. A further advantage of the scoring technique is that it gives abstracted information (score from 0 to 10) on the cardiovascular health status of a subject and therefore it can be easily interpreted by patients. However, it was shown that fuzzy logic had an important benefit for the implementation of the presented scoring concept due to the fact that fuzzy logic can be used to describe smooth transitions from normal to abnormal values and further from physiological to pathophysiological levels.

The setup procedure for surrogate variables of arterial stiffness (pPP, cPP, PWV and cAI) showed that there is still a lack of age-separated reference values for the normality and abnormality of surrogate variables. Therefore, the present reference values can be used as an additional reference for the future definition of normal and reference values.

Score results of each surrogate variable of arterial stiffness were investigated for the impact of different risk factors (smoking, obesity and DM II). An important finding of this investigation was that surrogate variables of arterial stiffness are differently influenced by each risk factor. Hence, weighting factors for the calculation of an overall score have to be chosen differently depending on the present risk factor. In conclusion, it was found that smoking has a fundamentally different impact on surrogate variables than obesity or DM II. Therefore, cAI is a good indicator for middle-aged (40-59 years) smokers but cAI has no informative value for obese subjects or subjects with DM II. In addition, pPP should not be used as indicator for young smokers. Furthermore, cPP and PWV can be used as indicator for each investigated risk factor over the whole investigated age range (40-79 years). Hence, it will be important for future guidelines to give information about which surrogate variables are more important as classification variables for different classes of age and present risk factors to improve screening procedures.

An important novel aspect of the present scoring system based on surrogate variables of arterial stiffness is that it gives no information related to cardiovascular risk of a subject. The present score reflects structural changes of the arterial system measured as a pathophysiological increase of arterial stiffness due to the impact of risk factors or genetic predisposition and it gives no information on the risk for having a future cardiovascular event. Hence, whereas risk scores give longitudinal information for future events, the present score gives cross-sectional information on the normality or abnormality of the variable at the time of measurement. In conclusion, this approach provides an additional methodology to the assessment of cardiovascular risk for the estimation of the cardiovascular health status of a subject. However, it is clear that further validation steps will be important for the

establishment of the technique. In addition, the implementation of further indicators or markers of the cardiovascular health status such as the measurement of the intima media thickness and the assessment of distensibility coefficients based on ultrasound techniques or the measurement of flow mediated dilatation and ankle-brachial-index could have an important additional benefit for the present scoring system.

In conclusion, the present scoring concept and scoring technique have been shown to be a promising novel approach and the scoring system based on easily measurable surrogate variables of arterial stiffness could be used in future after additional validation for screening procedures, preventive examinations or for the implementation into devices.

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11. Appendix

11.1. Summary statistics

11.1.1. ACCT raw data

Table 8: Summary statistics of ACCT raw data.

age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)					
subjects	M+F	n=368	n=728	n=1791	n=1481
	M	n=176	n=300	n=919	n=1025
	F	n=191	n=425	n=869	n=452
mean	M+F	46.2	51.2	57.1	65.3
	M	48.5	52.6	58.1	64.6
	F	44.2	50.3	56.2	66.9
SD	M+F	10.8	12.1	13.5	16.4
	M	9.5	11.3	13.1	15.9
	F	11.5	12.6	13.8	17.3
median	M+F	46.0	50.0	56.0	63.0
	M	48.0	51.5	56.0	63.0
	F	44.0	48.0	55.0	65.0
IQR	M+F	11.0	15.5	17.0	20.0
	M	10.0	15.0	17.0	20.0
	F	12.0	15.0	17.0	21.5
cPP (mmHg)					
subjects	M+F	n=340	n=651	n=1644	n=1371
	M	n=170	n=269	n=862	n=968
	F	n=169	n=379	n=781	n=400
mean	M+F	35.1	41.0	46.7	54.0
	M	34.7	40.5	46.3	52.8
	F	35.5	41.4	47.0	56.6
SD	M+F	8.8	10.8	12.4	15.9
	M	8.7	9.9	12.2	15.6
	F	9.0	11.3	12.6	16.2
median	M+F	34.0	40.0	45.0	52.0
	M	33.0	40.0	44.0	51.0
	F	35.0	40.0	45.0	55.0
IQR	M+F	11.0	15.0	16.0	19.0
	M	10.0	13.3	15.0	19.0
	F	11.0	15.0	15.0	19.5
PWV (m/s)					
subjects	M+F	n=366	n=707	n=1712	n=1413
	M	n=174	n=294	n=881	n=982
	F	n=191	n=410	n=829	n=427
mean	M+F	7.23	8.17	9.37	10.93
	M	7.48	8.45	9.68	11.14
	F	7.01	7.98	9.05	10.45
SD	M+F	1.29	1.75	2.27	2.78
	M	1.44	1.92	2.31	2.75
	F	1.08	1.57	2.18	2.80
median	M+F	7.03	7.88	8.95	10.43
	M	7.29	8.05	9.25	10.70
	F	6.84	7.72	8.64	9.98
IQR	M+F	1.50	2.00	2.58	3.39
	M	1.60	2.11	2.73	3.44
	F	1.37	2.00	2.41	3.14
cAI (%)					
subjects	M+F	n=359	n=711	n=1788	n=1474
	M	n=175	n=294	n=920	n=1021
	F	n=183	n=414	n=865	n=449
mean	M+F	23.5	28.8	30.1	30.8
	M	17.5	23.9	26.8	29.0
	F	29.1	32.3	33.6	34.9
SD	M+F	12.1	10.7	9.5	9.1
	M	10.7	10.0	9.1	8.8
	F	10.4	9.8	8.7	8.6
median	M+F	24.1	29.1	30.2	31.1
	M	17.1	23.6	26.8	29.5
	F	30.0	32.1	33.9	35.2
IQR	M+F	16.6	14.2	12.3	11.8
	M	14.0	13.9	11.8	11.6
	F	13.4	13.2	10.6	11.3

11.1.2. Reference group

Table 9: Summary statistics of pPP (mmHg) (reference group).

age group			40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
subjects		M+F	n=50	n=62	n=106	n=59
		M	n=16	n=28	n=43	n=39
		F	n=34	n=34	n=63	n=20
mean*		M+F	45.3	45.0	48.1	51.9
		M	49.4	47.1	47.6	50.9
		F	43.6	43.4	48.4	54.0
SD*		M+F	7.3	8.5	9.1	9.6
		M	5.1	8.0	7.4	8.9
		F	7.4	8.6	10.2	10.8
median		M+F	45.0	46.0	48.0	52.0
		M	50.0	46.5	48.0	51.0
		F	43.0	43.0	49.0	56.0
IQR		M+F	9.0	13.0	13.0	13.8
		M	6.5	12.0	11.5	8.8
		F	10.0	15.0	13.8	15.5
percentile	55%	M+F	47.0	46.0	49.0	53.0
	75%		50.0	53.0	55.0	59.8
	95%		59.0	59.8	65.0	72.0
	55%	M	50.0	48.8	51.0	52.0
	75%		52.5	54.0	53.8	54.8
	95%		59.1	61.5	57.4	69.4
	55%	F	44.0	44.2	49.0	58.0
	75%		49.0	52.0	56.0	62.0
	95%		58.4	56.8	65.7	72.0

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

Table 10: Summary statistics of cPP (mmHg) (reference group).

age group			40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
subjects		M+F	n=46	n=46	n=100	n=54
		M	n=13	n=20	n=40	n=38
		F	n=33	n=26	n=60	n=16
mean*		M+F	33.6	36.4	39.0	42.5
		M	32.7	36.8	37.5	40.9
		F	34.0	36.0	40.1	46.6
SD*		M+F	5.1	8.2	8.4	8.3
		M	3.1	7.9	6.5	7.5
		F	5.7	8.7	9.5	9.2
median		M+F	34.0	37.0	39.0	42.5
		M	32.0	37.0	39.0	40.0
		F	35.0	36.5	41.0	50.0
IQR		M+F	7.0	10.0	11.0	12.0
		M	3.8	9.0	8.5	10.0
		F	7.3	12.0	14.0	14.5
percentile	55%	M+F	34.0	37.8	40.0	43.2
	75%		37.0	41.0	45.0	49.0
	95%		42.8	51.2	54.0	59.0
	55%	M	32.7	37.5	39.0	41.4
	75%		34.5	41.0	42.5	47.0
	95%		39.6	51.0	46.0	55.0
	55%	F	35.0	37.8	41.0	51.3
	75%		37.0	43.0	48.0	53.5
	95%		45.4	52.0	57.5	60.7

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

Table 11: Summary statistics of PWV (m/s) (reference group).

age group			40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
subjects		M+F	n=50	n=61	n=105	n=57
		M	n=16	n=27	n=43	n=39
		F	n=34	n=34	n=62	n=18
mean*		M+F	6.65	7.56	7.98	9.18
		M	6.82	7.81	8.19	9.14
		F	6.57	7.37	7.84	9.25
SD*		M+F	0.83	1.21	1.63	1.78
		M	0.88	1.03	1.49	1.55
		F	0.81	1.31	1.70	2.27
median		M+F	6.78	7.46	7.89	9.00
		M	6.78	7.65	8.24	9.00
		F	6.73	7.26	7.80	8.99
IQR		M+F	1.04	1.39	1.86	1.91
		M	0.56	1.25	2.12	1.32
		F	1.23	1.30	1.78	3.88
percentile	55%	M+F	6.81	7.63	8.17	9.22
	75%		7.18	8.25	9.00	9.91
	95%		7.88	9.81	11.12	13.33
	55%	M	6.80	7.80	8.54	9.30
	75%		6.92	8.53	9.41	9.79
	95%		9.36	9.87	10.86	12.85
	55%	F	6.84	7.34	8.00	9.08
	75%		7.20	8.05	8.63	11.60
	95%		7.81	9.76	12.13	13.78

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

Table 12: Summary statistics of cAI (%) (reference group).

age group			40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
subjects		M+F	n=50	n=57	n=106	n=59
		M	n=16	n=26	n=43	n=39
		F	n=34	n=31	n=63	n=20
mean		M+F	21.4	26.9	30.7	29.9
		M	9.7	20.8	26.5	27.6
		F	26.8	32.0	33.5	34.3
SD		M+F	11.8	10.3	9.1	8.9
		M	7.8	8.6	7.8	7.6
		F	9.1	8.6	8.8	9.6
median		M+F	22.2	26.2	30.8	29.3
		M	9.9	21.1	24.6	28.0
		F	27.4	29.9	33.6	34.0
IQR		M+F	15.8	13.5	12.8	12.1
		M	8.0	12.5	10.8	10.3
		F	10.9	12.9	12.3	11.5
percentile	55%	M+F	23.8	27.7	31.5	30.0
	75%		28.5	34.4	36.0	36.0
	95%		38.0	42.3	47.3	45.8
	55%	M	10.1	22.6	27.6	28.7
	75%		13.3	26.0	31.4	31.9
	95%		25.1	35.4	41.4	41.5
	55%	F	27.8	32.2	34.4	35.8
	75%		31.5	38.4	40.0	40.5
	95%		40.4	49.7	48.1	50.1

11.1.2.1. PWV and cAI data confounder-corrected

Table 13: Summary statistics of PWV (m/s) (reference group; PWV data corrected for MAP = 95mmHg).

age group			40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
subjects		M+F	n=46	n=50	n=101	n=54
		M	n=13	n=21	n=40	n=38
		F	n=33	n=29	n=61	n=16
mean*		M+F	6.97	7.90	8.37	9.77
		M	7.16	8.06	8.53	9.57
		F	6.90	7.78	8.26	10.25
SD*		M+F	0.81	1.03	1.59	1.71
		M	0.97	0.83	1.39	1.53
		F	0.75	1.15	1.71	2.10
median		M+F	7.09	7.89	8.39	9.54
		M	7.32	8.10	8.73	9.44
		F	7.07	7.72	8.24	9.64
IQR		M+F	1.02	1.41	1.87	1.90
		M	1.05	0.98	1.81	1.70
		F	1.20	1.59	1.89	3.28
percentile	55%	M+F	7.11	8.08	8.63	9.69
	75%		7.50	8.52	9.23	10.52
	95%		8.05	9.99	11.80	14.04
	55%	M	7.40	8.17	8.85	9.66
	75%		7.62	8.46	9.45	10.13
	95%		9.50	9.71	11.04	13.30
	55%	F	7.09	7.73	8.43	9.77
	75%		7.50	8.57	9.17	12.10
	95%		8.03	10.06	12.21	14.73

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

Table 14: Summary statistics of cAI (%) (reference group; cAI data corrected for height = 1.70 m and HR = 65 bpm).

age group			40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
subjects		M+F	n=49	n=49	n=105	n=59
		M	n=16	n=23	n=43	n=39
		F	n=33	n=26	n=62	n=20
mean		M+F	22.6	24.9	29.8	29.1
		M	14.8	20.9	26.8	27.9
		F	26.3	28.4	31.8	31.4
SD		M+F	9.3	9.1	7.4	7.0
		M	7.0	8.3	6.7	6.0
		F	7.8	8.3	7.1	8.0
median		M+F	22.3	24.7	30.0	28.9
		M	15.6	22.0	26.5	28.0
		F	26.6	28.0	32.3	31.5
IQR		M+F	12.0	10.8	9.2	8.7
		M	11.1	9.0	7.6	7.9
		F	9.3	11.1	9.8	9.8
percentile	55%	M+F	24.2	25.4	31.2	29.2
	75%		28.7	31.0	34.1	34.0
	95%		36.7	41.5	41.0	42.4
	55%	M	16.5	22.8	27.1	28.7
	75%		19.7	26.3	31.6	31.9
	95%		26.2	33.6	37.7	37.9
	55%	F	28.3	28.2	33.0	32.2
	75%		30.4	33.2	37.5	35.8
	95%		37.4	45.7	44.5	44.6

11.1.3. Reference group with included hypertensive subjects

Table 15: Summary statistics of reference group with included hypertensive subjects.

age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)					
subjects	M+F	n=56	n=77	n=142	n=108
	M	n=18	n=35	n=70	n=74
	F	n=38	n=42	n=72	n=34
mean*	M+F	46.1	48.1	52.3	59.5
	M	50.7	48.7	54.3	58.6
	F	44.0	47.6	50.5	61.4
SD*	M+F	8.2	11.8	13.4	15.4
	M	6.3	9.2	14.5	15.4
	F	7.9	13.7	12.2	15.3
median	M+F	46.0	47.0	51.5	60.0
	M	50.0	49.0	54.0	58.5
	F	43.5	46.0	49.5	61.5
IQR	M+F	10.0	14.3	15.0	20.0
	M	8.0	11.8	14.0	19.0
	F	10.0	15.0	17.0	20.0
cPP (mmHg)					
subjects	M+F	n=52	n=57	n=133	n=101
	M	n=15	n=26	n=65	n=72
	F	n=37	n=31	n=68	n=29
mean*	M+F	34.4	38.3	42.6	49.2
	M	34.1	38.2	43.5	47.7
	F	34.5	38.5	41.8	53.1
SD*	M+F	6.0	9.9	12.1	13.8
	M	4.9	8.4	13.2	13.6
	F	6.5	11.2	11.0	13.4
median	M+F	34.0	38.0	42.0	49.0
	M	33.0	37.5	42.0	48.0
	F	35.0	38.0	41.0	53.0
IQR	M+F	7.5	14.8	13.3	18.5
	M	5.8	13.0	11.0	16.5
	F	9.3	16.3	14.5	15.3
PWV (m/s)					
subjects	M+F	n=56	n=77	n=141	n=106
	M	n=18	n=34	n=70	n=74
	F	n=38	n=43	n=71	n=32
mean*	M+F	6.73	7.61	8.35	10.05
	M	6.92	7.92	8.75	10.19
	F	6.64	7.38	7.97	9.74
SD*	M+F	0.85	1.38	1.77	2.37
	M	0.89	1.30	1.76	2.31
	F	0.83	1.40	1.69	2.48
median	M+F	6.80	7.47	8.35	9.71
	M	6.80	7.64	8.80	9.87
	F	6.82	7.30	8.07	9.32
IQR	M+F	1.07	1.63	2.14	3.31
	M	1.02	1.50	2.63	3.03
	F	1.22	1.68	1.69	4.21
cAI (%)					
subjects	M+F	n=56	n=71	n=142	n=108
	M	n=18	n=33	n=70	n=74
	F	n=38	n=38	n=72	n=34
mean	M+F	22.0	27.5	30.7	30.8
	M	10.7	21.9	27.8	29.0
	F	27.4	32.4	33.4	34.7
SD	M+F	11.6	9.9	8.7	8.3
	M	8.0	8.5	8.1	7.4
	F	8.9	8.2	8.3	8.7
median	M+F	22.8	27.1	30.3	31.0
	M	10.2	23.0	27.5	29.4
	F	27.6	31.5	33.5	36.5
IQR	M+F	15.4	13.5	11.3	11.3
	M	8.4	11.2	9.7	9.0
	F	12.3	11.5	11.1	10.9

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

11.1.4. Smoker group

Table 16: Summary statistics of smoker group.

age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)					
subjects	M+F	n=33	n=52	n=69	n=81
	M	n=17	n=19	n=45	n=72
	F	n=16	n=33	n=24	n=9
mean*	M+F	44.3	47.0	54.3	61.6
	M	47.4	45.7	55.6	61.4
	F	41.2	47.8	52.1	63.4
SD*	M+F	9.3	9.9	11.9	12.1
	M	11.0	8.0	13.4	11.8
	F	6.5	10.9	8.9	15.4
median	M+F	45.0	46.0	53.0	60.0
	M	48.0	45.0	54.0	59.0
	F	42.5	48.0	52.5	63.0
IQR	M+F	10.5	11.5	10.3	14.5
	M	11.0	9.8	10.3	14.0
	F	10.0	12.5	11.0	14.5
cPP (mmHg)					
subjects	M+F	n=27	n=46	n=66	n=78
	M	n=16	n=15	n=43	n=70
	F	n=11	n=31	n=23	n=8
mean*	M+F	34.8	37.6	44.1	50.0
	M	34.8	35.7	44.4	50.0
	F	34.8	38.6	43.6	50.7
SD*	M+F	9.3	8.9	10.7	12.3
	M	10.4	5.8	12.0	11.8
	F	8.2	10.3	8.3	17.6
median	M+F	36.0	38.0	43.5	49.0
	M	33.5	36.0	43.0	49.0
	F	38.0	41.0	45.0	47.5
IQR	M+F	9.8	12.0	10.0	16.0
	M	10.0	9.5	9.8	15.0
	F	9.0	11.8	8.8	26.5
PWV (m/s)					
subjects	M+F	n=34	n=52	n=67	n=78
	M	n=17	n=19	n=43	n=70
	F	n=17	n=33	n=24	n=8
mean*	M+F	6.85	7.90	9.23	10.62
	M	6.73	8.18	9.77	10.85
	F	6.97	7.75	8.34	8.82
SD*	M+F	0.92	1.81	2.09	2.49
	M	0.91	2.29	2.15	2.34
	F	0.93	1.53	1.65	2.81
median	M+F	6.80	7.92	9.11	10.63
	M	6.53	8.08	9.81	10.88
	F	7.11	7.78	8.40	8.40
IQR	M+F	1.15	2.23	2.95	3.31
	M	0.93	2.10	3.44	3.05
	F	0.89	2.28	2.03	2.13
cAI (%)					
subjects	M+F	n=29	n=49	n=68	n=81
	M	n=16	n=17	n=44	n=72
	F	n=13	n=32	n=24	n=9
mean*	M+F	26.5	29.6	31.6	30.3
	M	21.3	26.0	28.7	30.6
	F	33.0	31.6	36.8	28.3
SD*	M+F	14.2	9.5	9.1	8.0
	M	9.7	9.6	8.4	7.5
	F	16.0	8.9	8.1	10.6
median	M+F	28.2	31.0	31.8	31.7
	M	20.0	28.5	29.5	31.6
	F	35.4	31.6	36.3	33.0
IQR	M+F	18.8	15.6	9.9	11.5
	M	15.2	14.5	9.3	11.1
	F	14.9	13.3	11.2	14.3

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

11.1.5. Obesity group

Table 17: Summary statistics of obesity group

age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)					
subjects	M+F	n=45	n=60	n=98	n=53
	M	n=26	n=18	n=35	n=30
	F	n=19	n=42	n=63	n=23
mean*	M+F	44.4	51.3	53.5	61.8
	M	46.1	50.0	53.7	62.3
	F	42.2	51.9	53.4	61.1
SD*	M+F	11.9	12.2	13.7	16.0
	M	10.7	8.6	11.1	16.6
	F	13.0	13.7	15.1	15.5
median	M+F	45.0	51.0	54.0	62.0
	M	45.5	51.5	54.0	63.5
	F	45.0	49.0	54.0	60.0
IQR	M+F	10.0	18.0	17.0	17.8
	M	10.0	8.0	17.0	20.0
	F	11.8	22.0	14.0	14.3
cPP (mmHg)					
subjects	M+F	n=44	n=54	n=93	n=48
	M	n=26	n=16	n=34	n=29
	F	n=18	n=38	n=59	n=19
mean*	M+F	32.8	40.2	43.1	50.1
	M	32.1	37.0	42.4	49.0
	F	33.9	41.6	43.5	51.7
SD*	M+F	10.3	10.9	12.4	16.0
	M	9.7	7.6	10.4	17.2
	F	11.3	12.1	13.6	14.1
median	M+F	33.5	39.0	44.0	49.0
	M	31.0	38.5	40.5	49.0
	F	34.5	44.0	45.0	52.0
IQR	M+F	12.0	18.0	14.5	15.5
	M	14.0	7.5	15.0	20.0
	F	9.0	19.0	14.5	11.5
PWV (m/s)					
subjects	M+F	n=44	n=58	n=94	n=52
	M	n=25	n=18	n=34	n=30
	F	n=19	n=40	n=60	n=22
mean*	M+F	7.33	7.82	9.67	10.55
	M	7.56	7.65	9.90	11.76
	F	7.03	7.90	9.55	9.10
SD*	M+F	1.23	1.59	2.34	4.40
	M	1.12	0.84	2.36	2.84
	F	1.32	1.86	2.34	5.16
median	M+F	7.19	7.99	9.25	10.70
	M	7.46	7.80	9.25	12.43
	F	6.80	8.08	9.25	9.35
IQR	M+F	1.85	1.80	2.47	4.10
	M	1.80	1.23	2.33	3.87
	F	1.91	2.17	2.62	2.77
cAI (%)					
subjects	M+F	n=45	n=57	n=97	n=53
	M	n=26	n=18	n=35	n=30
	F	n=19	n=39	n=62	n=23
mean*	M+F	21.8	26.7	29.4	30.0
	M	17.7	22.0	26.3	26.9
	F	27.4	28.9	31.1	34.0
SD*	M+F	10.0	9.8	8.3	8.1
	M	8.6	9.8	8.6	7.3
	F	9.0	8.9	7.7	7.2
median	M+F	20.0	27.2	29.0	30.1
	M	16.6	22.2	25.4	27.9
	F	30.0	27.8	32.2	35.7
IQR	M+F	15.7	10.7	11.4	12.0
	M	12.1	14.0	10.3	10.7
	F	17.5	10.5	10.2	11.6

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

11.1.6. Diabetes mellitus II group

Table 18: Summary statistics of diabetes mellitus II group

age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)					
subjects	M+F	n=12	n=34	n=86	n=70
	M	n=8	n=23	n=71	n=57
	F	n=4	n=11	n=15	n=11
mean*	M+F	45.8	54.7	58.0	67.3
	M	47.3	54.0	59.0	65.1
	F	43.0	56.4	53.7	77.4
SD*	M+F	12.9	12.0	16.4	16.7
	M	11.0	11.5	15.2	15.7
	F	17.7	13.4	20.9	18.5
median	M+F	47.5	52.5	57.0	66.5
	M	47.5	51.0	57.0	63.0
	F	43.5	54.0	57.0	72.0
IQR	M+F	18.5	21.0	22.0	24.0
	M	17.0	18.0	24.0	21.8
	F	25.5	20.0	14.5	25.0
cPP (mmHg)					
subjects	M+F	n=11	n=34	n=84	n=66
	M	n=8	n=23	n=68	n=53
	F	n=3	n=11	n=16	n=11
mean*	M+F	33.4	41.7	44.7	53.4
	M	32.3	39.4	44.9	51.1
	F	36.7	47.0	43.7	62.8
SD*	M+F	9.9	9.9	15.1	17.7
	M	8.8	8.4	14.2	16.8
	F	15.1	11.7	19.2	18.6
median	M+F	31.0	42.5	44.5	53.0
	M	31.0	42.0	44.0	50.0
	F	44.0	43.0	46.5	59.0
IQR	M+F	18.8	11.0	21.5	24.0
	M	16.5	13.8	22.5	24.0
	F	17.3	20.5	14.0	18.8
PWV (m/s)					
subjects	M+F	n=12	n=31	n=84	n=65
	M	n=8	n=22	n=68	n=52
	F	n=4	n=9	n=16	n=11
mean*	M+F	8.07	8.84	10.41	11.41
	M	8.63	9.04	10.47	11.44
	F	7.04	8.38	10.14	11.46
SD*	M+F	2.38	2.58	2.78	2.67
	M	2.94	3.00	3.02	2.79
	F	0.87	1.49	1.68	2.46
median	M+F	7.79	8.47	10.10	11.55
	M	8.48	8.87	10.15	11.67
	F	7.00	8.47	10.04	11.39
IQR	M+F	1.98	3.68	4.06	3.37
	M	3.79	4.09	4.66	3.70
	F	1.40	1.25	2.25	2.58
cAI (%)					
subjects	M+F	n=11	n=34	n=87	n=70
	M	n=8	n=23	n=70	n=57
	F	n=3	n=11	n=17	n=11
mean*	M+F	21.4	24.2	26.1	27.7
	M	17.9	20.4	25.4	26.6
	F	30.7	32.1	29.0	31.9
SD*	M+F	13.5	9.8	9.8	9.1
	M	13.1	9.1	9.9	9.1
	F	9.5	5.5	8.7	8.2
median	M+F	17.3	24.4	27.6	28.5
	M	15.3	20.6	26.8	27.5
	F	37.3	31.1	28.9	33.3
IQR	M+F	25.6	14.3	16.1	12.7
	M	18.7	10.7	16.2	12.4
	F	15.1	11.5	10.2	9.5

*the natural logarithm was taken of each dataset (mean and SD are back-transformed)

11.2. Reference values

11.2.1. Uncorrected reference values

Table 19: Reference values calculated based on the reference group (reference value calculation rule 1).

	age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)	normal	M+F	50	51	54	58
		M	52	52	52	57
		F	48	49	56	62
	high	M+F	57	59	63	67
		M	57	60	59	65
		F	55	58	65	72
cPP (mmHg)	normal	M+F	37	42	45	48
		M	34	42	42	46
		F	38	42	47	53
	high	M+F	42	50	53	56
		M	37	50	48	53
		F	43	51	56	62
PWV (m/s)	normal	M+F	7.1	8.3	9.1	10.4
		M	7.3	8.4	9.2	10.2
		F	7.0	8.2	9.1	10.9
	high	M+F	7.9	9.5	10.7	12.1
		M	8.1	9.4	10.6	11.6
		F	7.8	9.5	10.7	13.1
cAI (%)	normal	M+F	32	35	38	37
		M	17	28	33	33
		F	34	39	40	42
	high	M+F	43	45	46	45
		M	24	36	40	41
		F	43	47	49	51

Table 20: Reference values calculated based on the reference group (reference value calculation rule 2).

	age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)	normal	M+F	46	46	49	53
		M	50	48	49	52
		F	45	45	50	55
	high	M+F	57	59	63	68
		M	58	60	60	65
		F	56	58	65	72
cPP (mmHg)	normal	M+F	34	37	40	44
		M	33	38	38	42
		F	35	37	41	48
	high	M+F	42	50	53	56
		M	38	50	48	53
		F	43	50	56	62
PWV (m/s)	normal	M+F	6.8	7.7	8.2	9.4
		M	6.9	7.9	8.4	9.3
		F	6.7	7.5	8.1	9.5
	high	M+F	8.0	9.6	10.7	12.1
		M	8.3	9.5	10.6	11.7
		F	7.9	9.5	10.7	13.0
cAI (%)	normal	M+F	23	28	32	31
		M	11	22	28	29
		F	28	33	35	36
	high	M+F	41	44	46	45
		M	23	35	39	40
		F	42	46	48	50

Table 21: Reference values calculated based on the reference group (reference value calculation rule 3).

	age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)	normal	M+F	50	53	55	60
		M	53	54	54	55
		F	49	52	56	62
	high	M+F	59	66	68	73
		M	60	65	65	64
		F	58	66	70	79
cPP (mmHg)	normal	M+F	37	41	45	49
		M	35	41	43	47
		F	37	43	48	54
	high	M+F	45	52	56	61
		M	38	51	52	55
		F	46	55	62	72
PWV (m/s)	normal	M+F	7.2	8.3	9.0	9.9
		M	6.9	8.5	9.4	9.8
		F	7.2	8.0	8.6	11.6
	high	M+F	8.3	9.5	10.7	11.9
		M	7.6	9.5	11.4	11.0
		F	8.6	9.2	10.5	14.8
cAI (%)	normal	M+F	29	34	36	36
		M	13	26	31	32
		F	31	38	40	40
	high	M+F	46	46	50	47
		M	22	40	41	43
		F	44	49	52	51

Table 22: Reference values calculated based on the reference group (reference value calculation rule 4).

	age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
pPP (mmHg)	normal	M+F	47	46	49	53
		M	50	49	51	52
		F	44	44	49	58
	high	M+F	59	60	65	72
		M	59	62	57	69
		F	58	57	66	72
cPP (mmHg)	normal	M+F	34	38	40	43
		M	33	38	39	41
		F	35	38	41	51
	high	M+F	43	51	54	59
		M	40	51	46	55
		F	45	52	58	61
PWV (m/s)	normal	M+F	6.8	7.6	8.2	9.2
		M	6.8	7.8	8.5	9.3
		F	6.8	7.3	8.0	9.1
	high	M+F	7.9	9.8	11.1	13.3
		M	9.4	9.9	10.9	12.8
		F	7.8	9.8	12.1	13.8
cAI (%)	normal	M+F	24	28	31	30
		M	10	23	28	29
		F	28	32	34	36
	high	M+F	38	42	47	46
		M	25	35	41	41
		F	40	50	48	50

11.2.2. Confounder-corrected reference values

Table 23: Reference values for PWV and cAI calculated based on the confounder-corrected reference group data.

	age group		40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
Reference values calculated by reference value calculation rule 1						
PWV (m/s)	normal	M+F	7.4	8.5	9.5	10.9
		M	7.7	8.4	9.4	10.5
		F	7.3	8.5	9.5	11.7
	high	M+F	8.2	9.5	11.0	12.5
		M	8.6	9.2	10.8	12.0
		F	8.0	9.6	11.1	13.7
cAI (%)	normal	M+F	30	32	35	34
		M	21	28	32	32
		F	32	35	37	37
	high	M+F	39	41	42	41
		M	27	36	38	38
		F	40	43	44	45
Reference values calculated by reference value calculation rule 2						
PWV (m/s)	normal	M+F	7.1	8.0	8.6	10.0
		M	7.3	8.2	8.7	9.8
		F	7.0	7.9	8.5	10.5
	high	M+F	8.3	9.6	11.0	12.6
		M	8.7	9.4	10.8	12.1
		F	8.1	9.7	11.1	13.7
cAI (%)	normal	M+F	24	26	31	30
		M	16	22	28	29
		F	27	29	33	32
	high	M+F	38	40	42	41
		M	26	35	38	38
		F	39	42	44	45
Reference values calculated by reference value calculation rule 3						
PWV (m/s)	normal	M+F	7.5	8.5	9.2	10.5
		M	7.6	8.5	9.4	10.1
		F	7.5	8.6	9.2	12.1
	high	M+F	8.6	10.0	11.2	12.4
		M	8.9	9.6	11.4	12.0
		F	8.9	10.1	11.1	14.6
cAI (%)	normal	M+F	29	31	34	34
		M	20	26	32	32
		F	30	33	38	36
	high	M+F	40	41	44	42
		M	32	36	38	40
		F	41	45	47	46
Reference values calculated by reference value calculation rule 4						
PWV (m/s)	normal	M+F	7.1	8.1	8.6	9.7
		M	7.4	8.2	8.8	9.7
		F	7.1	7.7	8.4	9.8
	high	M+F	8.0	10.0	11.8	14.0
		M	9.5	9.7	11.0	13.3
		F	8.0	10.1	12.2	14.7
cAI (%)	normal	M+F	24	25	31	29
		M	17	23	27	29
		F	28	28	33	32
	high	M+F	37	42	41	42
		M	26	34	38	38
		F	37	46	44	45

11.3. Regression equations

11.3.1. PWV regression lines

Table 24: Regression equations for PWV (m/s) depending on MAP (mmHg).

reg. equation		$PWV = a_0 + a_{MAP} * MAP$			
age group		**40-49 yrs**	**50-59 yrs**	**60-69 yrs**	**70-79 yrs**
ACCT data					
a_0	M+F	2.81	2.74	2.11	3.63
	M	2.61	3.34	2.35	4.13
	F	3.38	2.79	2.33	2.76
a_{MAP}	M+F	0.047	0.057	0.075	0.073
	M	0.051	0.053	0.074	0.070
	F	0.039	0.055	0.070	0.078
R^2	M+F	0.20	0.13	0.14	0.10
	M	0.18	0.09	0.13	0.10
	F	0.21	0.16	0.13	0.11
p-value	M+F	p<0.001	p<0.001	p<0.001	p<0.001
	M	p<0.001	p<0.001	p<0.001	p<0.001
	F	p<0.001	p<0.001	p<0.001	p<0.001
F-stat	M+F	87.33	101.02	262.97	155.89
	M	35.96	25.55	129.84	103.71
	F	46.12	72.81	112.93	48.70
reference group					
a_0	M+F	4.05	4.07	2.45	6.94
	M	3.72	0.82	1.63	7.86
	F	4.54	6.06	3.33	-3.43
a_{MAP}	M+F	0.030	0.040	0.063	0.029
	M	0.036	0.078	0.074	0.017
	F	0.024	0.016	0.053	0.153
R^2	M+F	0.12	0.10	0.08	0.02
	M	0.12	0.36	0.13	0.01
	F	0.09	0.02	0.05	0.13
p-value	M+F	p<0.05	p<0.05	p<0.01	p=0.366
	M	p=0.242	p<0.01	p<0.05	p=0.588
	F	p=0.087	p=0.509	p=0.086	p=0.168
F-stat	M+F	6.15	5.29	8.74	0.83
	M	1.53	10.85	5.78	0.30
	F	3.13	0.45	3.05	2.11
reference group with included hypertensive subjects					
a_0	M+F	4.51	4.07	3.04	2.62
	M	4.24	2.93	3.85	1.96
	F	4.74	4.82	3.57	4.69
a_{MAP}	M+F	0.025	0.040	0.058	0.078
	M	0.031	0.054	0.052	0.085
	F	0.021	0.030	0.050	0.057
R^2	M+F	0.17	0.13	0.15	0.21
	M	0.16	0.15	0.15	0.27
	F	0.16	0.11	0.07	0.09
p-value	M+F	p<0.01	p<0.01	p<0.001	p<0.001
	M	p=0.146	p<0.05	p<0.01	p<0.001
	F	p<0.05	p=0.054	p<0.05	p=0.124
F-stat	M+F	9.91	9.20	23.70	26.62
	M	2.39	4.71	11.47	25.64
	F	6.54	3.99	5.10	2.52

11.3.2. cAI regression lines

Table 25: Regression equations for cAI (%) depending on HR (bpm) and body height (m).

reg. equation		$cAI = a_0 + a_{HR} * HR + a_{height} * height$			
age group		**40-49 yrs**	**50-59 yrs**	**60-69 yrs**	**70-79 yrs**
		ACCT data			
a_0	M+F	156.3	138.0	129.7	117.8
	M	146.7	89.1	104.0	89.6
	F	74.9	128.4	98.8	112.8
a_{HR}	M+F	-0.391	-0.415	-0.455	-0.402
	M	-0.378	-0.442	-0.480	-0.412
	F	-0.375	-0.390	-0.429	-0.404
a_{height}	M+F	-63.23	-48.74	-41.67	-36.09
	M	-59.22	-20.60	-26.63	-19.69
	F	-12.91	-43.23	-22.78	-31.87
R^2	M+F	0.32	0.30	0.35	0.37
	M	0.25	0.25	0.32	0.34
	F	0.15	0.21	0.26	0.31
p-value	M+F	p<0.001	p<0.001	p<0.001	p<0.001
	M	p<0.001	p<0.001	p<0.001	p<0.001
	F	p<0.001	p<0.001	p<0.001	p<0.001
F-stat	M+F	80.0	144.0	471.3	417.4
	M	27.7	44.7	214.4	258.1
	F	15.5	49.5	147.8	96.7
		reference group			
a_0	M+F	174.2	130.0	129.6	131.2
	M	113.1	38.7	102.0	123.1
	F	72.8	112.4	111.2	109.0
a_{HR}	M+F	-0.438	-0.432	-0.520	-0.362
	M	-0.181	-0.535	-0.504	-0.364
	F	-0.416	-0.419	-0.573	-0.372
a_{height}	M+F	-73.24	-44.55	-38.89	-45.20
	M	-50.67	9.25	-24.16	-40.68
	F	-10.90	-32.73	-24.86	-30.45
R^2	M+F	0.42	0.26	0.38	0.37
	M	0.11	0.23	0.29	0.33
	F	0.23	0.16	0.39	0.27
p-value	M+F	p<0.001	p<0.001	p<0.001	p<0.001
	M	p=0.431	p<0.05	p<0.001	p<0.001
	F	p<0.05	p=0.084	p<0.001	p<0.001
F-stat	M+F	18.82	10.39	42.88	30.55
	M	0.89	3.89	13.41	17.71
	F	5.19	2.70	21.35	5.60