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Use of non-invasive imaging methods to assess unexpected cardiovascular risk using hypertension as test of concept

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Eidesstattliche Versicherung

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Ich erkläre hiermit an Eides statt,

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Abstract

Introduction: High arterial stiffness (AS) and loss of capillary density (CD) are biomarkers of cardiovascular risk. This study assessed whether combined measurement of AS and CD improves assessment of cardiovascular risk beyond that predicted by classical scoring systems.

Subjects & Methods: 150 outpatients with treated hypertension were studied (means ± SEM: age 62.6 ± 1.2 years, blood pressure (Omron) 146/87 ± 2/1 mmHg, female 41), with hypercholesterolemia in 77, diabetes mellitus in 32, chronic kidney disease in 34, ischemic heart disease in 41, stroke syndromes in 27 and peripheral vascular disease in 47 patients. AS was estimated from aortic pulse wave velocity (PWV); basal (BCD) and maximum skin capillary density (MCD) were estimated by capillary video microscopy. Readings of PWV $\geq$ 10 m/s, BCD $\leq$ 59 per 0.6 mm^2 field and MCD $\leq$ 66/field were regarded as abnormal, indicating pathological AS and capillary rarefaction, respectively. Written informed consent was obtained for the studies, which were approved by the local research ethics committee.

Results: Readings were obtained both for arterial stiffness (PWV 10.8 ± 0.3 m/sec) and capillary density (BCD 54 ± 1 / field; MCD 62 ± 1/ field) in 100 patients (67%: 95% CI 95 - 105), for PWV alone (10.6 ± 0.63 m/sec) in 18 patients (12%: 95% CI 4 - 32), for capillary density alone (BCD 50 ± 2/field; MCD 58 ± 2.16/ field) in 24 patients (16%: 95% CI 11 - 37); and neither measurement in 8 patients (3%: 95%CI 0 - 19). Both PWV and capillary density were abnormal in 42 patients (42 %: 95%CI 30 - 54). Capillary density was low, but PWV normal in 32 patients (32 %: 95%CI 18 - 46). PWV was raised, but capillary density normal in 18 patients (18%: 95% CI 11-30); both readings were normal in 7 patients (7%: 95%CI 1-13). The QRISK2 risk score, a common cardiovascular (CV) risk score for patients without CV diseases, showed a highly significant correlation with PWV (r_s=0,443; p=0,001). In contrast, the Pocock score, a CV risk score for patients with CV co-morbidities, correlated inversely with BCD (r_s= -0,253; p= 0,039). PWV correlated positively with systolic blood pressure (r_s=0.270, p=0.004), pack years (r_s=0.242, p=0.008) and age (r_s=0.335, p<0.001). BCD correlated highly with MCD (r_s=0.869, p<0.001). Of the 52 patients in the subgroup with low-to-intermediate CV risk 40% showed abnormally high PWV and 73% abnormally low BCD and respectively 61% low MCD. There was no significant correlation between PWV and BCD or MCD, before or after adjustment for history of ischemic heart disease.

Conclusions: The high incidence of pathological readings for AS (40%), BCD (over 70%) and MCD (over 60%) among patients with low CV risk score shows severe unexpected vascular damage in healthy subjects. Therefore, these parameters help to identify additional patients at increased CV risk beyond the prediction by classical CV risk factors alone. Large and small vessel readings were only concordant in 2 out of 5 patients in indicating increased cardiovascular risk. Capillary imaging identified 15 percent of patients as having an increased cardiovascular risk not detected by PWV alone. These findings support use of diagnostic tests for both micro- and macrocirculation when assessing clinical cardiovascular risk in patients with hypertension with or without cardiovascular comorbidity.

Zusammenfassung

Verwendung nichtinvasiver Darstellungsmethoden zur Beurteilung des kardiovaskulären Risikos unter Verwendung von Bluthochdruck als Anwendungsbeispiel

Einleitung: In dieser Studie wurden die Verringerung der Kapillardichte (KD), zu Englisch Capillary Rarefaction (CR), sowie die arterielle Steifheit (engl. Arterial Stiffness/ AS) als neue Biomarker zur Beurteilung des kardiovaskulären (KV) Risikos von Patienten mit arterieller Hypertension verwendet. Verringerte KD, in dieser Arbeit am Beispiel des dermalen Gefäßbettes demonstriert, ist als Biomarker assoziiert mit den Hauptrisikofaktoren für KV-Erkrankungen. AS bezeichnet den Verlust der physiologischen arteriellen Elastizität durch arteriosklerotischen Umbau. Dieser zeigt sich in der Zunahme der Pulswellengeschwindigkeit (engl. Pulse Wave Velocity/ PWV), da der Windkesseleffekt mit sinkender arterieller Compliance abnimmt. Erhöhte AS hat als unabhängiger KV Risikofaktor Einzug in aktuelle Leitlinien zur Vorhersage des Erkrankungsrisikos an KV Erkrankungen gefunden. Diese Studie untersuchte, ob die kombinierte Erhebung von AS und KD die Vorhersage von individuellem KV Risiko bei Patienten mit arterieller Hypertonie verbessert im Vergleich zur alleinigen Beurteilung durch klassische KV Risikofaktoren.

Hypothese: Die kombinierte Erfassung von AS und KD verbessert die Abschätzung des individuellen KV Risikos über die Vorhersage durch klassische Risk Scores hinaus.

Studienteilnehmer: Volljährige Patienten mit diagnostizierter Hypertonie mit oder ohne KV Vorerkrankung nach schriftlicher Einverständniserklärung in Studienteilnahme sowie Zustimmung durch das örtliche Ethikkomitee für Forschung. **Methoden: Risk Score:** Erfassung der klassischen KV Risikofaktoren sowie Berechnung des individuellen statistischen Risikos eines KV-Ereignisses in den nächsten 10 Jahren (für Patienten ohne bekannte KV Vorerkrankung : QURISK2-Score, für Patienten mit bekannter KV Vorerkrankung: Pocock-Score). **Kapillardichte:** Visualisierung der dermalen Kapillaren am Fingerrücken in Vivo per Auflichtmikroskop (KK Technology). Beobachtung von vier Gesichtsfelder (GF, je 0.6 mm^2) pro Patient für je eine Minute unter Ruheperfusion zur Bestimmung der funktionellen basalen KD (BCD). Bestimmung der strukturellen maximalen KD (MCD) nach zweiminütiger venöser Stauung des Fingers per Miniaturmanschette. Als pathologisch wurde eine BCD $\leq$ 59 / GF und MCD $\leq$ 66 / GF gewertet.
Arterielle Steifheit: Bestimmung der PWV per Tensio Clinic Arteriograph. Als patho-

logisch hoch wurde eine PWV ≥ 10 m/s gewertet. Anschließender Vergleich des durch den Score berechneten Risikos mit den Werten für KD und AS.

Ergebnisse: Es wurden 150 ambulante Patienten, davon 41 Frauen, mit behandelter Hypertonie untersucht (mittleres Alter: 62,6 ± 1,2 Jahre, mittlerer Blutdruck (Omron): 146/87 ± 2/1 mmHg). 82 Patienten hatten KV Vorerkrankungen. Bei 100 Patienten (67%: 95% CI 95 - 105) konnten Ergebnisse für PWV (10,8 ± 0.3 m/s) und KD (BCD 54 ± 1 / GF; MCD 62 ± 1/ GF) gewonnen werden, bei 18 Patienten (12%: 95% CI 4 - 32) nur PWV (10.6 ± 0.63 m/s), bei 24 Patienten (16%: 95% CI 11 - 37) nur KD (BCD 50 ± 2/GF; MCD 58 ± 2,16/ GF) und bei 8 Patienten (3%: 95%CI 0 - 19) weder AS noch KD. Bei 42 Patienten waren sowohl PWV als auch KD pathologisch (42 %: 95%CI 30 - 54). In 32 Fällen war die KD niedrig, aber die PWV normal (32 %: 95%CI 18 - 46). Eine erhöhte PWV in Kombination mit normaler KD zeigte sich bei 18 Patienten (18%: 95% CI 11-30) und in 7 Fällen waren beide Parameter normal (7%: 95% CI 1-13). Der QRISK2-Score korrelierte positiv mit PWV (r_s=0,443; p=0,001), der Pocock-Score negativ mit BCD (r_s= -0,253; p= 0,039). PWV korrelierte positiv mit systolischem Blutdruck (r_s=0.270, p=0.004), Pack Years (r_s=0.242, p=0.008) und Alter (r_s=0.335, p<0.001). BCD korrelierte signifikant mit MCD (r_s=0.869, p<0.001). Von den 52 Patienten in der Untergruppe mit niedrigem bis mittlerem KV Risiko zeigten 40% pathologisch hohe PWV und 73% bzw. 61% pathologisch niedrige BCD und MCD. PWV und BCD korrelierten nicht signifikant, weder vor, noch nach Adjustierung für KV Vorerkrankungen.

Zusammenfassung: Die hohe Inzidenz pathologischer Ergebnisse für BCD (über 70%), MCD (über 60%) und AS (über 40%) unter Patienten mit niedrigem CV Risk Score offenbarte schwerwiegende unerwartete Schädigung des Gefäßsystems gesunder Probanden. Deswegen zeigt diese Studie, dass diese Parameter zusätzliche Patienten unter erhöhtem KV Risiko identifizieren, im Vergleich zu alleiniger Beurteilung durch klassische KV Risikofaktoren. Des Weiteren ergänzt KD den etablierten Biomarker AS bei der Identifizierung von Patienten mit Mikro- und Makroangiopathie. Dies zeigt sich an den Ergebnissen für AS und KD, die nur in 2 von 5 Patienten übereinstimmend erhöhtes KV Risiko zu erkennen gaben. Kapilläre Bildgebung identifizierte in dieser Studie ein Drittel an Patienten unter erhöhtem KV Risiko, das durch alleinige Messung von AS nicht erfasst worden wäre. Ein Kostenvoranschlag basierend auf den finanziellen, personellen und zeitlichen Ressourcen dieser Studie zeigt außerdem, dass KD kostengünstig (zu 4 bis 10 ₺ pro Test) bestimmt werden könnte und durch Früherkennung und intensivere Therapie gefährdeter Personen hohe Folgekosten und Spätschäden durch KV Ereignisse verringern könnte.

Abbreviations

ADMA	Asymmetric Dimethyl Arginine
AIx	Augmentation Index
AS	Arterial Stiffness
BCD	Basal Capillary Density
BP	Blood Pressure
CD	Capillary Density
CI	Confidence Interval
CKD	Chronic Kidney Disease
CR	Capillary Rarefaction
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
FCR	Functional Capillary Rarefaction
FRS	Framingham Risk Score
HDL	High Density Lipoprotein
HTN	Hypertension
MCD	Maximum Capillary Density
NO	Nitric Oxide
PWV	Pulse Wave Velocity

1. Background

1.1 Hypertension

According to the world health statistics of May 2012, every third adult worldwide suffers from raised blood pressure (BP). The highest percentage is found in developing countries where hypertension (HTN) is poorly diagnosed and treated [1]. The WHO considered the importance of this topic by devoting the World Health Day 2013 to HTN [2].

HTN or high BP is a chronic medical condition in which the arterial BP is permanently raised above a reading of 140 mmHg systolic or 90 mmHg diastolic as defined by the WHO. Table 1 shows the classification of different stages of HTN [3].

Table 1: Classification of Different Stages of Hypertension

Blood Pressure [mmHg]	**systolic**	**diastolic**
normal	90 – 119	60 – 79
PreHTN	120 – 139	80 – 89
HTN stage 1	140 – 159	90 – 99
HTN stage 2	>160	>100
Systolic HTN	>140	normal

Around 90 – 95 % of cases of HTN is idiopathic and is considered the consequence of genetic, metabolic and lifestyle factors since no singular medical explanation for the raised BP has been found. A smaller percentage of approximately 5 – 10% has an underlying, treatable condition such as renal artery stenosis or endocrine diseases like thyroid disorders, hyperaldosteronism, Cushing syndrome and/or phaeochromocytoma. Obstructive sleep apnea and a coarctation of the aorta may also cause secondary HTN [4].

It is widely accepted that the BP level correlates strongly with the development of cardiovascular disease (CVD) leading to stroke, myocardial infarction, and chronic kidney injury [5]. Blood pressure and CVD are strongly correlated to each other, i.e. the higher the BP the more likely stroke, myocardial infarction or chronic kidney disease (CKD) become [3]. Furthermore, the necessity of BP lowering treatment has to be considered individually for each patient, taking into account all additional cardiovascular risk factors. Hence, a pa-

tient with multiple risk factors may receive antihypertensive medication at an earlier stage than someone without comorbidities [3].

1.2 Cardiovascular Risk Factors

Many different conditions have been found to increase the risk for cardiovascular diseases in individuals. Table 2 lists the major CV risk factors contributing to the development of CVD [6]. Risk factors can be split into modifiable and non-modifiable ones. The risk factors that contribute most to CVD are mainly modifiable. Hence lifestyle changes have a big influence on BP control and can help to decrease or avoid drug treatment. However, often these steps do not improve BP control sufficiently so that antihypertensive drugs are needed. Most of the major risk factors found their way into different risk scores [7-9] that are currently used to assess the probability of a cardiovascular event in a specific person during a certain period of time, normally calculated for ten years.

Table 2: Cardiovascular Risk Factors

	Major risk factors	Other risk factors
Modifiable	– Smoking – HTN – Hypercholesterolemia (high low density lipoprotein, low high density lipoprotein (HDL) cholesterol) – Diabetes mellitus (DM) – Patient history of CVD – Left ventricular hypertrophy – Proteinuria	– Overweight – High caloric and fatty nutrition – Physical inactivity – Impaired lipid metabolism – Impaired fasting glucose – Chronic inflammation / raised C-reactive Protein
Non-modifiable	Increasing age	– Direct relatives with premature stroke, myocardial infarction, peripheral vascular disease, DM or HTN – Ethnicity – Male sex

1.3 Cardiovascular Risk Scores

1.3.1 Risk Score Approach

Due to the high incidence of HTN and its CV sequelae in nowadays societies, a great necessity of useful tools has arisen to detect and treat patients that are at risk of HTN and its complications, such as ischemic heart disease, stroke, congestive cardiac failure, peripheral arterial disease and chronic renal disease. The challenge to filter these patients out before they suffer any sequelae from elevated BP has been the primary objective of cardiovascular risk scores.

Several scores have been developed in the past decades to predict the individual risk of a person to suffer adverse cardiovascular events, especially myocardial infarction and stroke. Table 3 offers an overview of the characteristics of each risk score at the end of this chapter.

All of the common risk scores include age, sex, systolic BP, cholesterol levels (most of them also HDL level) and smoking history as major risk factors for a CV event. Most CV risk scores classify patients according to their risk factors into three groups: Patients with less than 10% for a CV event during the next 10 years are considered as low risk, 10 – 20% correspond to intermediate CVD risk and more than 20% during the next 10 years to high CVD risk. Every patient with a high CVD risk requires lifestyle changes under professional medicinal supervision and management of the underlying risk factors[10].

Some scores also include parameters that might imply additional CV risk. For example, the level of high-sensitivity C-reactive protein correlates with the low grade systemic inflammation which accompanies arteriosclerosis [11]. Also, homocysteine levels, afro-Caribbean ethnicity, family history for myocardial infarction/stroke, abdominal obesity and social deprivation have been related to an increased CV risk [12]. Also the exclusion of certain groups, such as patients with diabetes mellitus, and the development of extra scoring systems for this kind of patients has been shown to increase the significance of these screening tools [13]. This is explained by the adaption of the score to the higher CV risk of these patients due to their comorbidity which is often underestimated by less elaborate scores [9].

The following sections give more detailed insight into different scoring systems that are currently being used in the United States, the United Kingdom and Germany:

1.3.2 Framingham Risk Score

The Framingham Risk Score (FRS) derives from the Framingham Heart Study that was started in 1948 in Framingham, United States, to investigate the influence of different factors such as diet and exercise on the development of cardiovascular diseases. Meanwhile, the third generation of participants has joined the study, providing a huge long-term amount of data which makes the Framingham Heart Study one of the largest ongoing studies about cardiovascular risks worldwide. Based on the findings of the study the first risk score was published in 1998 as an algorithm to predict the risk of coronary heart disease taking in account various risk factors. Over the years several changes have been undertaken on the FRS in such way that today various outcomes (cardiovascular disease, stroke, coronary disease, myocardial infarction and death from either coronary or cardiovascular disease) over various time periods can be calculated [14]. However, various studies suggest that the FRS overestimates CV-risk especially when it is used for populations that differ from the original cohort in the United States, e.g. European countries [15,16].

1.3.3 JBS2

The JBS2 has been designed based on the guidelines of the Joint British Societies, a consortium of six societies for cardiovascular diseases [10]. Due to its publication in the British National Formulary, the standard drug reference in the United Kingdom, it has found widespread usage in medical practice. The aim of the JBS2 is "to reduce the risk of a non-fatal or fatal atherosclerotic cardiovascular event and to improve both quality and length of life" in clinical practice.[10]. The guidelines focus especially on patients with any established form of CVD, asymptomatic people with a high risk score (20% or higher for CVD in the next 10 years) and patients with diabetes. Additionally, persons who show a single risk factor that is especially elevated should receive CVD prevention. This includes elevated BP > 160 mm Hg systolic or > 100 mm Hg diastolic, or lesser degrees of BP elevation with target organ damage, elevated total cholesterol to HDL cholesterol ratio > 6.0, familial dyslipidaemia, such as familial hypercholesterolemia or familial combined hyperlipidaemia and people with a family history of premature CVD [10]. It is important to know that the JBS2 score does not include the criteria of diabetes and left ventricular hypertrophy as does the FRS, because patients with DM and left ventricular hypertrophy are automatically considered high risk by the JBS2 score.

1.3.4 HeartScore

The HeartScore is the risk score developed by the European Society of Cardiology. In order to render the score more accurate in its international application, it has been adapted to different European countries by discerning two groups (high and low risk countries). Furthermore, it is available as a calibrated version for several European countries. This fine tuning may be the reason why it has outperformed the FRS in several studies [17].

There are two existing versions: The Full Score which takes into account age, gender, smoking status, BP and lipids. If lipid parameters are not available, a simplified BMI Score with age, gender, smoking status and BMI can be used as an approximation for the CVD risk in the meanwhile [7].

1.3.5 Pocock Score

The Pocock score is based on the analysis of eight randomised controlled trials of antihypertensive treatment, including 47.008 patients of whom 3001 died. It calculates the risk of death from CVD in the next five years, including death from stroke and coronary heart disease [18].

Most risk scores regard a patient with diagnosed CVD (i.e. manifestation of stroke, myocardial infarction, peripheral vascular disease) as high risk. However, the Pocock score is the only score which currently allows some differentiation in patients with a positive medical history for CVD as it takes into account the patient's past medical history of LVH, DM, stroke, and myocardial infarction, and adds them as an additional risk for future CVDs. For this reason the Pocock score was used in this study to calculate the CV risk for patients with a diagnosed CVD.

1.3.6 UKPDS

The UKPDS risk engine is a scoring system based on the data of the United Kingdom Prospective Diabetes Study which was performed between 1977 and 1997 [9]. In addition to classical risk factors, this score adds the duration of DM and the level of HbA1c to the risk algorithm. These adaptions make allowance to the higher CV risk in patients with DM which is often underestimated by scores as for example the FRS because this score includes only a small number of diabetic subjects [9].

1.3.8 QRISK2

This score is an updated version of the QURISK score and was validated in a prospective open cohort between 1993 and 2008, including 2.3 million patients in England and Wales and is based on over 16 million patients years [19]. Apart from classical risk factors such as systolic blood pressure, cholesterol, diabetes and family history for coronary heart disease, this score also considers other aspects such as ethnicity, atrial fibrillation (AF), rheumatoid arthritis, CKD, BMI and current antihypertensive treatment. Furthermore, it includes a wider age range (25 – 84 years) and a more sophisticated smoking history than most scores and is updated regularly. Therefore this score was used to calculate cardiovascular risk in the participants of this study who had no previously diagnosed CVD. The calculator as well as further information regarding QURISK2 can be found under www.qrisk.org.

1.3.9 Differences among Cardiovascular Risk Equations

To resume the characteristic features of the above named risk scores Table 3 shows their similarities and differences.

Table 3: Similarities and Differences of Cardiovascular Risk Scores

	Framingham (FRS)	**JBS2**	**SCORE / HeartScore**	**Pocock Score**	**UKPDS risk engine**	**QRISK2**
Calculation	-Different time periods (4 – 12 years) -Different outcomes (CVD, stroke, coronary heart disease, myocardial infarction and death from either coronary heart disease or CVD)	-10 years risk -Cardiovascular risk based on the sum of coronary heart disease and stroke risk of FRS	-10 years risk	-5 years risk -Risk of death from CVD (including stroke and coronary heart disease)	- Risk for fatal and non-fatal stroke and coronary heart disease	-10 years risk -Risk for myocardial infarction or stroke
Data source	US	UK	Europe	UK	UK	UK
Age range	35 to 75	35 to 75 (restricted to 3 age groups (<50, 50 to 59 and >60 years)	20 to 100			25 to 84
Sex	Yes	Yes	Yes	Yes	Yes	Yes
Systolic. BP	Yes	Yes	Yes	Yes	Yes	Yes
Cholesterol	Yes	Yes	Yes	Yes	Yes	Yes

HDL	Total cholesterol: HDL ratio	Total cholesterol: HDL ratio	Total HDL	No	Total HDL	Total cholesterol: HDL ratio
Smoking	Binary (yes/no)	Binary (yes/no)	Binary (yes/no)	Yes	Yes	Yes (5 groups)
Diabetes	Yes, incorporation of DM as a risk factor	No, as patient with DM is considered as high risk	No	Yes	Yes (very detailed)	Yes (none/ type 1/ type 2)
LVH	Yes	No, as patient with left ventricular hypertrophy is considered as high risk	No	Yes	No	No
Patient History for CVD	No	No	No	Yes	No	No
Special Feature	-Can measure risk for different outcomes and time periods	-Exclusion of patients with left ventricular hypertrophy or DM	-Adjusted to different European countries - Division between high and low risk countries -Full score and BMI score available	-Measures height and creatinine -History of stroke /MI -Includes also patients with CV comorbidities	-Ethnicity, AF, DM type 2, duration, HBA1C -Separate outcomes for fatal and non-fatal stroke and coronary heart disease	- Ethnicity, AF, rheumatoid arthritis, CKD, BMI and current antihypertensive treatment -sophisticated smoking history (non /ex/ light/moderate/heavy)

The above mentioned CV risk scores classify patients according to their risk factors into three groups:

Low CVD risk (<10% during the next 10 years); Intermediate CVD risk (10 – 20% during the next 10 years); High CVD risk (>20% during the next 10 years)

1.4 Vascular Biomarkers

1.4.1 Arterial Stiffness

Thomas Young was the first to predict the connection between pulse wave velocity (PWV), heart work and arterial stiffness (AS) in his Croonian Lecture in 1808 [20]. Since then, many studies, including an evaluation of the Framingham study cohort in 2010 [21], have suggested that elevated AS, indicated by a high PWV, is closely connected with increased CV risk [22,23]. A major step in this development has been the declaration of high AS to be an independent risk factor for CV events by the European Heart Society in 2006 [24].

In this study the reading of the PWV and of the augmentation index of the aorta (AIx) are used to assess AS. The AIx is calculated by the ratio of the systolic pulse wave P_1 and the reflected pulse wave P_2.

1.4.1.1 Definition of Arterial Stiffness

AS describes the artery's ability to expand during systole and contract during diastole. Common terms related to the elastic properties of the artery are explained in Table 4.

Table 4: Terms Related to Vascular Properties

Term	Meaning
Elasticity	Property of a material to return to its originals shape after deformation [25]
Compliance	Change in volume per change in pressure [26]
Strain	Deformation of a body relative to its original length [25]
Stress	Force applied to a unit of a surface [25]
Elastic modulus	Tendency of a material to deform under applied force; = stress/strain [25]
Pulse Pressure	Difference between systolic and diastolic blood pressure [27]

1.4.1.2 Pathophysiology of Arterial Stiffness

The process of arterial stiffening causes negative consequences for the body, especially for the CV system. Physiologically, the aorta and the other big elastic arteries of the body expand at the ejection of blood during the systole and thus accommodate approximately 50% of the stroke volume. Subsequently, the vessel is passively restored to its original width by its elastic properties [28]. This so-called "Windkessel effect" reduces heart work and causes continuous forward blood flow. The loss of arterial compliance decreases the Windkessel effect. The artery no longer accommodates under the influence of systolic pressure. This generates more resistance for the heart to eject the blood and causes higher demand of energy, respectively oxygen. In the course of time this causes LVH, thus further increasing myocardial oxygen demand, risk for myocardial hypoxemia, subsequent fibrosis and left ventricular failure [29]. Due to the loss of the arterial cushioning effect, BP rises higher during the systole and only returns to normal levels during the diastole. Therefore it is called isolated systolic HTN. This phenomenon is especially found in elderly people aged 60 and above [30]. The spreading of the pulse wave along a stiff arterial tree is several folds faster than in a healthy artery. This causes an early reflection of the pulse wave, which clashes with the original pulse wave at an earlier point of the systole and causes an augmentation of systolic pressure. In combination with the loss of shock absorption this high systolic pressure penetrates stronger into arterioles and end organs and damages them by mechanic impact [31] as well as by induction of pro-inflammatory stimuli [32], causing for example in the kidneys chronic renal failure due to increased glomerular sclerosis [33].

Figure 1: Windkessel Effect in Large Elastic Artery

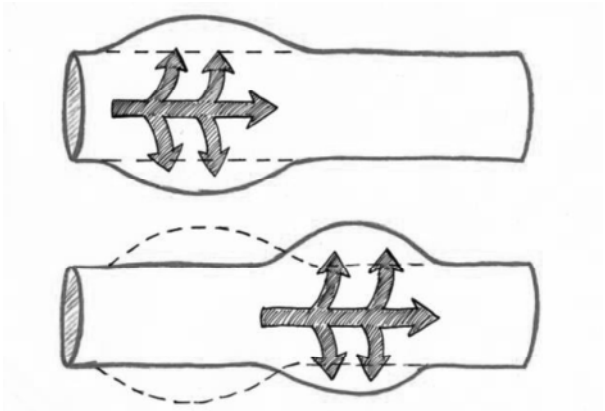
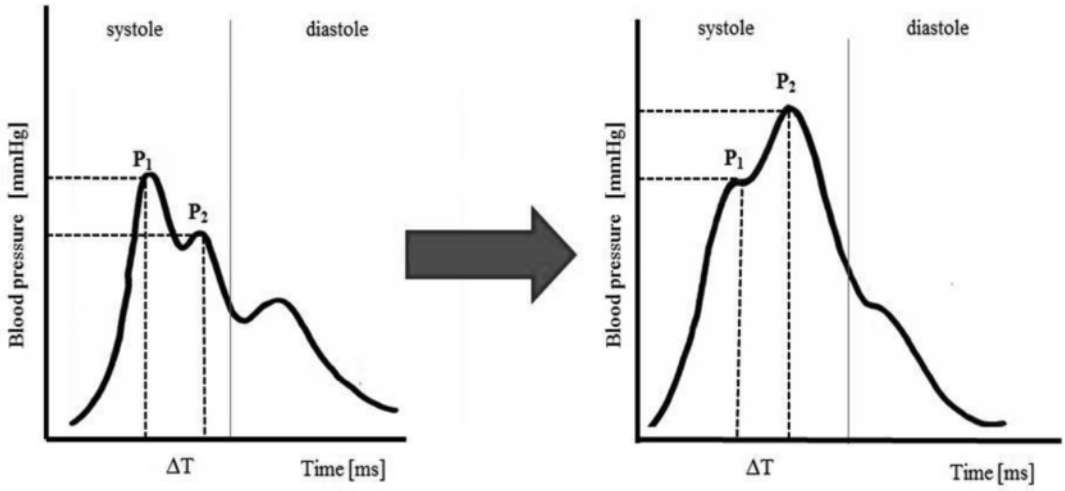


Figure 2: Reflection of Pulse Wave in Artery

a) Physiologic Elasticity b) Increased Stiffness

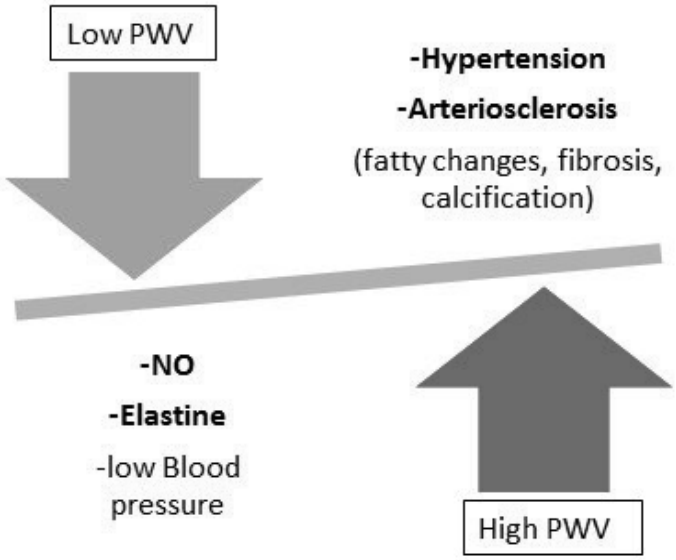


P_2 arrives during late systole in healthy elastic artery. P_2 arrives during early systole in stiff artery (shorter ΔT), increasing systolic blood pressure and left ventricular work-load

1.4.1.3 Major Risk Factors for Arterial Stiffness

AS is mainly influenced by the structure and function of the arterial wall and by haemodynamics within the artery. The major CV risk factors all contribute to increase AS.

Figure 3: Balance between Beneficial and Adverse Factors on Arterial Pulse Wave Velocity



1.4.1.3.1 Hypertension

HTN increases AS through several mechanisms. Firstly, there is an immediate effect of high BP on the aorta and the other big elastic arteries of the body. HTN causes the arteries to pre-stretch so that they provide less elastic reservoir during the systole. The arteries become stiffer. Therefore this effect should always be considered as a variable component in the calculation of the AS in individuals with HTN.

Secondly, HTN induces structural changes of the vessel wall. The increased shear stress damages the vascular endothelium which then fails in its complex functions such as vasodilatation through production of nitric oxide (NO) [34] and control of smooth muscle growth [35]. This lack of inhibition and the steady adaption of the vessels to the high intravascular pressure lead towards hypertrophy of vascular smooth muscle cells and subsequently to narrowing of the lumen and a more rigid vessel wall [36].

Thirdly, reduced bioavailability of NO causes a functional stiffening of the artery. Additionally, shear stress also induces the production of reactive oxygen species in endothelial cells [37] which oxidize BH_4 (tetrahydrobiopterine) to BH_2 (dihydrobiopterine). BH_4, L-arginine and oxygen are substrates used by the NO synthase (NOS) to produce NO. The oxidation of BH_4 to BH_2 by reactive oxygen species renders the molecule useless as a co-factor for synthesis of NO. Furthermore, NOS with BH_2 as a co-factor produces superoxide (O_2^-) instead of NO. Every condition that reduces NO bioavailability impairs vasodilatation and subsequently increases AS. Furthermore, reactive oxygen species not only damage cell components, producing oxidised low density lipoprotein and DNA defects, they also increase fibrosis inside the vessel wall as they oxidise fibroblast growth factor which activates fibroblasts to produce collagen[38]. Fibroblast growth factor activates the migration of smooth muscle cells within the vessel wall, which also contributes to arterial stenosis[39]. Thus, reactive oxygen species not only increase the loss of elasticity due to fibrosis but also intensify the whole arteriosclerotic process in the vessel wall, because of the accumulation of oxidised low density lipoprotein, growth of vascular smooth muscle cells, and induction of inflammation, which lead to changes in lipid composition and cell damage.

Fourthly, the underlying causes for secondary HTN can also influence AS through diverse effects on the cardiovascular system: In renal arterial stenosis the production of renin is pathologically high due to hypoperfusion of the stenosed kidneys. The activation of the renin-angiotensin-aldosterone-system causes extensive vasoconstriction, which increases the total peripheral resistance. The release of aldosterone results in sodium retention and secondary plasma volume expansion. The latter mechanism also plays a role in hyperaldosteronism.

Figure 4: Synthesis of Nitric Oxide under Normal Conditions

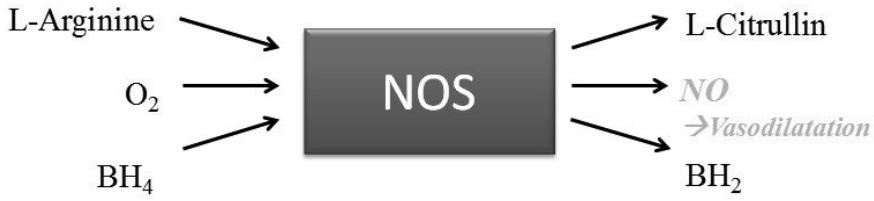
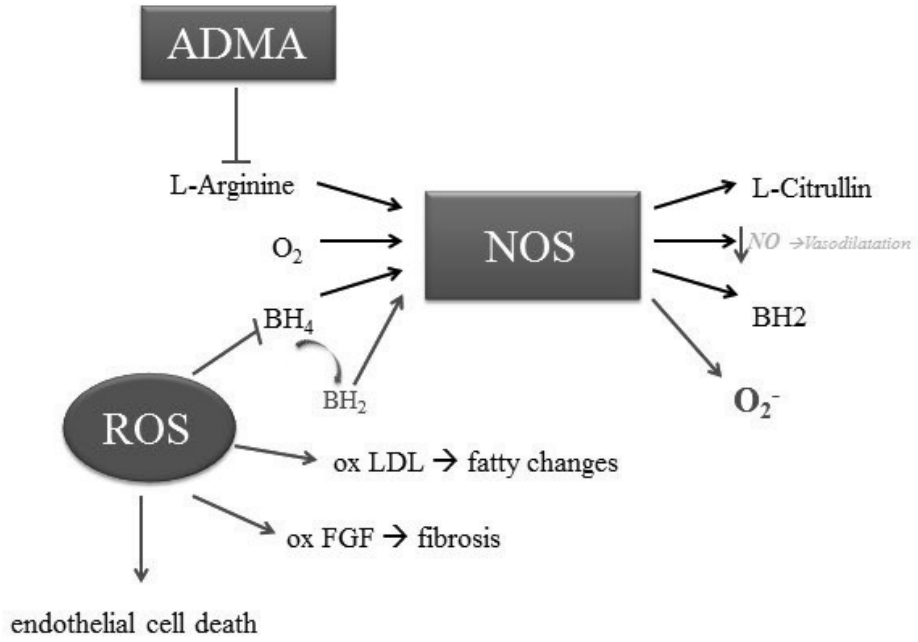


Figure 5: Effect of Elevated Levels of ADMA and Reactive Oxygen Species on Nitric Oxide-Synthetase



O_2= Oxygen

NO= Nitric Oxide

NOS= NO-Synthetase

ADMA= Asymmetric Dimethylarginine

ROS= Reactive Oxygen Species

BH_4= Tetrahydrobiopterin

BH_2=Dihydrobiopterin

1.4.1.3.2 Hypercholesterolaemia

Low density lipoprotein plays a central role in the development of arteriosclerosis [40]. Its oxidised or minimally modified derivatives stimulate pro-inflammatory signals in endothelial cells [41] or attract monocytes directly [42]. The latter then differentiate to macrophages. Due to the phagocytosis of oxidised low density lipoprotein, these macrophages then transform into foam cells. The core of the arteriosclerotic plaque consists of accumulated apoptotic foam cells. These plaques cause a loss in arterial elasticity. In patients with hypercholesterolaemia these processes seem to occur at an increased level which is supported by findings of elevated AS in these patients [43,44]. On the other hand, cholesterol-lowering statin therapy reduces AS [45,46].

1.4.1.3.3 Smoking

Smoking has multiple adverse effects on the arterial elastic properties, such as the production of reactive oxygen species (which disturb NO production in vascular endothelial cells), increase of cell adhesion and pro-inflammatory markers such as CRP, interleukin 6 and tumour necrosis factor α, by altering the lipid profile towards higher total cholesterol and lower HDL levels and by a rise in BP due to activation of the sympathetic nervous system [47]. All these changes are closely linked to increased AS. Thus, it is not surprising that smoking has shown to be significantly related to high AS [48]. An increase in AS can already be shown after a relatively short period of regular smoking in otherwise healthy subjects[49].

1.4.1.4 Other Cardiovascular Risk Factors

Other CV risk factors have been connected to increased AS, too, as shown by the following studies.

1.4.1.4.1 Gender

AS in pre-pubertal women is higher than in men but decreases with the onset of puberty and rises again after the menopause. Due to the vasoprotective and anti-inflammatory properties of oestrogen on the vascular system, this sex hormone is likely to be a major endocrine contributor to differences in AS found between men and pre-menopausal women [50,51]. It has been suggested that women have a higher constitutional AS which is lowered by the vasoprotective effects of oestrogen [52] during their reproductive years. These effects include an increase of endothelium-dependent [53] as well as -independent [54] vasodi-

latation, decrease of inflammatory response to intravascular injury and of CRP-dependent neointima formation [55]. All these factors help to maintain or respectively increase the elasticity of arteries.

1.4.1.4.2 Diabetes

Hyperglycaemia and oxidative stress in patients with DM yield an increased formation of advanced glycation end products [56]. This non-enzymatic reaction between sugars and proteins or lipids also takes place in the arterial wall, where advanced glycation end products create irreversible crosslinking between collagen and elastin molecules [57], rendering the arterial wall more rigid [58]. Advanced glycation end products also increase intravasal inflammation through binding to their receptors [59] and stimulation of reactive oxygen species formation [60], both of which promote arteriosclerosis. Accordingly, drugs that break these cross-links (e.g. alagebrium) have a positive effect on AS [61].

1.4.1.4.3 Chronic Kidney Disease

A loss in incretory renal function causes a lack of vitamin D which is needed for calcium homoeostasis. Hypocalcaemia stimulates the secretion of parathormone, which indirectly recruits new osteoclasts and promotes the release of calcium from bone tissue. The loss of excretory renal function on the other side leads to hyperphosphataemia. This brings about the precipitation of calcium-phosphate complexes in the tunica media [62]. This arterial media calcification decreases arterial compliance and increases PWV in patients with CKD [63].

Additionally, CKD patients show increased levels of ADMA [64]. This molecule is very similar to L-arginine and therefore inhibits and inactivates the NOS in competition to L-arginine. This decreases NO bioavailability [65]. CKD is a condition of elevated oxidative stress, which inhibits dimethyl arginine dimethylaminohydrolase 1 and 2 [66]. These enzymes degrade ADMA. Due to the inhibition of these enzymes, ADMA levels rise [66]. NO bioavailability in CKD is also decreased due to the loss of renal mass [67]. NOS can especially be found in the macula densa of the renal glomeruli which are located in the renal cortex [68]. Hence a loss of renal cortex deprives the body of an important source of NO-production.

1.4.2 Capillary Rarefaction

The phenomenon capillary rarefaction (CR) describes the decrease of capillaries in different tissues of the body. CR can be quantified by the measurement of capillary density (CD). CD is measured by the number of capillaries found per visual field in the capillary bed of a tissue. It can be assessed by capillary video microscopy, a method to study skin capillaries intravitally through detection of red blood cells in blood vessels. If CD falls under a certain pathological threshold it is considered as CR. The dermal capillary microcirculation offers an easy accessible, ready at hand surrogate to assess the systemic condition of capillary microcirculation [69,70]. A detailed description of capillary video microscopy can be found in the methods chapter.

In 1933 Rüdemann discovered CR by demonstrating pathologically low numbers of capillaries in the human retina [71]. Owing to studies conducted in the last two decades there is now increasing evidence showing a close connection between CR and major cardiovascular risk factors like HTN [72-74], diabetes [75], chronic kidney disease [76] and predisposing conditions like obesity, as well as CV diseases like ischemic heart disease or cardiomyopathy. Nowadays, CR is an established feature of these diseases. CR has since been found in many vital tissues such as retina [72], skin [73], heart [77] and kidneys [78].

1.4.2.1 Pathophysiology of CR

The loss of capillaries causes pathological changes both at the systemic and local level of the body. In reference to the cardiovascular system, CR decreases the cross-sectional area of the vascular bed which in turn increases the total peripheral resistance [73]. In the microcirculation, increased pressure on fewer capillaries (capillary HTN) enhances transcapillary hyperfiltration [79] and microvascular flow disturbances [80]. This disturbance of blood flow deteriorates the supply of oxygen and nutrients for the tissue. According to this theory, Levy et al state that "microvascular rarefaction will tend to both reduce the vessel surface […] available for oxygen delivery and increase the diffusional distance between vessels and their target cells. The resulting ischemia may be responsible for much of the end-organ damage associated with HTN" [81].

1.4.2.2 Mechanisms of CR

There are two different concepts regarding the development of CR. Structural capillary rarefaction describes the anatomical loss and therefore permanent absence of capillaries in the vascular bed [73]. This theory is supported by findings of reduced absolute numbers of capillaries in hypertensive patients [83]. The absolute numbers of capillaries (maximum capillary density; MCD) can be shown under venous congestion, when due to the restriction of venous outflow all capillaries in the vascular bed are filled with erythrocytes and therefore become visible under the capillary video microscope [84].

Functional capillary rarefaction (FCR) implies an impaired recruitment of existing, non-perfused capillaries. A method often applied to prove functional capillary rarefaction is post-occlusive reactive hyperaemia. which describes the effect of increased tissue perfusion after a period of ischemia, i.e. caused by occlusion of a limb. The accumulation of metabolic products and the decrease in oxygen saturation act as vasodilating stimuli, which increase capillary recruitment under normal conditions. Hypertensive subjects for example show fewer perfused capillaries, i.e. lower CD, compared to normotensive subjects under post-occlusive reactive hyperaemia [85].

Prewitt et al were the first to propose a chronological order of functional and structural capillary rarefaction in which adverse conditions like HTN cause local myogenic response and vasoconstriction in the microcirculation up to the point of non-perfusion of single capillaries equivalent to FCR. As a consequence of chronic constriction, capillaries obliterate and undergo attrition, which leads to secondary structural rarefaction [31].

1.4.2.3 Risk Factors associated with Capillary Rarefaction

1.4.2.3.1 Obesity

A negative correlation of capillary recruitment and visceral adiposity has already been observed in healthy individuals, showing that functional capillary rarefaction develops progressively with obesity [86,87]. Especially the capillary function appears to be impaired in obese subjects. This poses a possible link between obesity and impaired glucose metabolism, as insulin increases its own delivery and glucose uptake through increase of NO production and subsequent recruitment of additional capillary beds [88]. Capillary recruitment in the basal state and during hyperinsulinaemia is significantly attenuated in obese individuals [89], as well as in response to physiological hyperinsulinaemia after a meal [90]. In patients with metabolic syndrome highly reduced capillary reserve has been reported, too [91].

1.4.2.3.2 Diabetes

Many aspects of structural and functional CR in diabetes show similarities to conditions of capillary impairment in obese subjects. Diabetic subjects, just alike metabolic syndrome patients exhibit blunted capillary recruitment following meal-induced hyperinsulinaemia, observed by CD at baseline and under post-occlusive reactive hyperaemia. These findings were associated with impaired insulin sensitivity and postprandial hyperglycaemia [92]. FCR is already under way at an early stage of diabetes, detectable in young diabetic patients without comorbidities [93]. The lack of functional capillary reserve in these patients suggests that capillary dysfunction considerably precedes the onset of diabetic sequelae. Impairment of capillary function also promotes diabetic cardiomyopathy and strongly affects prognosis of CVD. These patients show elevated readings for markers of endothelial damage such as circulating endothelial cells and membrane microparticles. Myocardial CR leads to myocyte loss and fibrosis, promoting diabetic cardiomyopathy. Additionally, a low myocardial blush grade after percutaneous coronary intervention in diabetic patients with coronary artery disease is consistent with impaired myocardial microcirculation in diabetics [82]. Capillary function is impaired by poor metabolic control of DM, especially in the feet, and correlates with late microvascular complications although the macrovasculature is still intact [75,94]. This implies that CR is most likely to be both, cause and consequence of diabetes.

1.4.2.3.3 Hypertension

CR is an established feature of hypertensive disease [31,72,95]. However, the question whether CR plays an aetiological role in the development of HTN or is rather a consequence of it has not been finally settled.

Noon et al published findings of increased minimum resistance and low CD in young male with familial predisposition for HTN, proposing an influence of genetically impaired angiogenesis on the development of HTN [96]. Reduced CD in normotensive patients with positive family history for HTN support Noon's findings of a decreased angiogenic capacity of the microcirculation in individuals predisposed to HTN [97]. Early structural changes that preceded sustained HTN have also been shown in mild-borderline hypertensive patients [74]. Similar tests with spontaneously hypertensive rats also showed reduced CD antedating sustained HTN [98].

However, HTN itself causes local myogenic response and vasoconstriction in the microcirculation, up to the point of non-perfusion of single capillaries. Two studies with patients with pre-HTN /HTN stage 1 (cut-off 130 mmHg systolic blood pressure) found no

differences in structural CD compared to normotensive controls. However, both studies confirmed significant FCR, shown by low CD under post-occlusive reactive hyperaemia, being in line with the hypothesis of Prewitt et all that functional capillary rarefaction due to HTN induced vasoconstriction precedes structural capillary rarefaction caused by obliteration of non-perfused capillaries [31, 99, 100].

1.4.2.3.4 Chronic Kidney Disease

In CKD, local changes in the renal capillary bed as well as systemic changes in CD can be observed. Thang et al reported on lower skin CD in predialysis and dialysis patients at baseline, during post-occlusive reactive hyperaemia and venous congestion, indicating systemic functional and structural CR in CKD patients. Capillary parameters were inversely correlated with high serum levels for phosphorus and bicarbonate, suggesting an impact of the disturbed mineral metabolism in CKD on the integrity of the capillary network.[101] In the renal microvasculature, loss of peritubular capillaries is an important hallmark of CKD. Rarefaction of these peritubular capillaries brings about hypoxia in the kidney, an organ which is particularly susceptible to hypoxia due to its relatively low oxygen tension in the medulla caused by counter-current in this tissue [76]. Fibrosis and inflammation induced by hypoxia cause renal scarring, increase diffusion distances for oxygen, destroy more capillaries and worsen hypoxia [102]. Areas of low peritubular CD showed significantly higher macrophage infiltration as a sign of interstitial inflammation in biopsies of CKD kidneys [103]. Persistent loss of peritubular capillaries after acute kidney injury could initiate this vicious circle, explaining partly, why after acute kidney injury patients are at high risk to develop CKD [104]. Taken together, CR in CKD shows that vascular damage is likely to be a systemic feature, contributing to the initiation and aggravation of this disease.

1.4.3 Combined Assessment of Cardiovascular Risk by Classical Risk Scores, Arterial Stiffness and Capillary Density

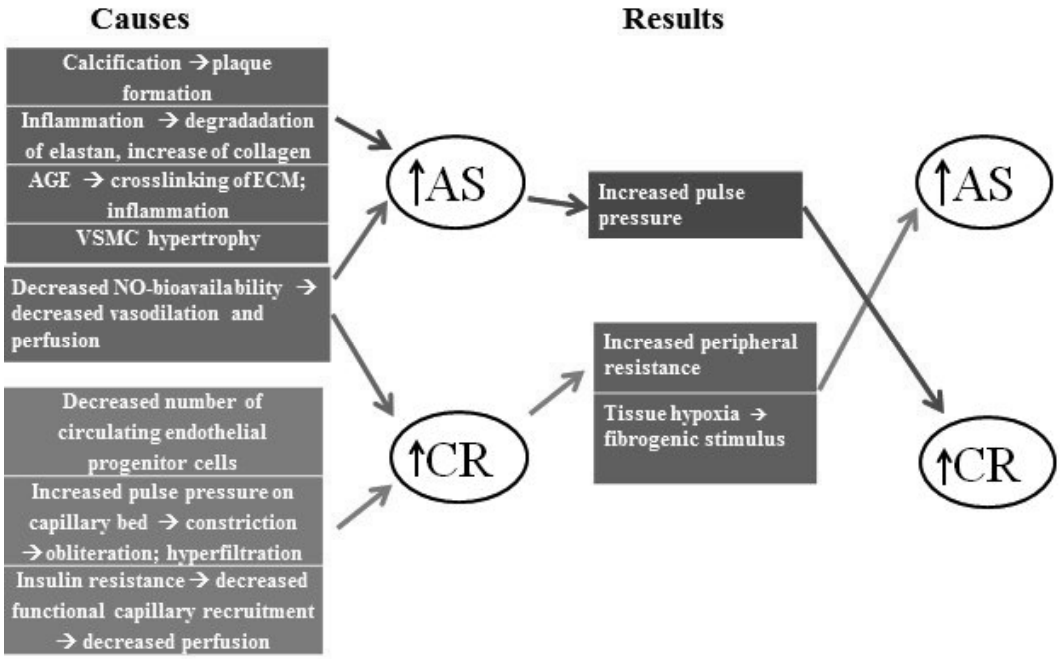
In the endeavour to assess CV risk, classical scores face the following dilemma. Underestimation of the personal risk exposes the patient to higher risk of adverse CV events due to the lack of addressing these risk factors appropriately. Overestimation and the implementation of vigorous drug therapy subject patients to unnecessary adverse drug effects such as bleeding, dizziness, postural hypotension and falls. Especially when applied to populations that differ from the original study cohort for which the risk score was designed, scores do not always reflect the actual risk for CV events correctly [15,17,105]. Both, AS and CR have been shown to be closely correlated with adverse CV outcomes [22,106] and might be useful tools to improve CV risk assessment. Important CV risk factors such as HTN, DM, smoking and CKD influence both factors, AS and CR, as described previously. However, one single risk factor has different respective effects on macro- compared to microvasculature.

The effects of HTN on large elastic arteries cause hypertrophy of vascular smooth muscle cells and intravascular inflammation inducing degradation of elastin and stimulation of fibrosis by production of collagen. In the capillary bed in turn, HTN leads to hyperfiltration, constriction and obliteration of the capillaries. The decrease in NO bioavailability deteriorates arterial elasticity as well as capillary perfusion and hence exerts an effect on both, micro- and macrovasculature.

Diabetes mellitus increases AS by the promotion of fibrosis and arteriosclerosis, production of advanced glycation end products and subsequent crosslinking of the extracellular matrix, whereas the toxic effects of chronic hyperglycaemia include endothelial damage, thickening of the basal membrane and osmotic swelling of the cells.

The results of increased AS and CR intertwine and mutually aggravate each other. Stiff arteries conduct pulse pressure without shock absorption into the capillary beds of end organs, increasing local shear stress. A decrease in CD poses a higher peripheral resistance to the heart, increasing BP and consequently AS. Tissue hypoxia due to CR acts as a strong fibrotic stimulus, deteriorating both CD and arterial elasticity. The following figure depicts the interactions of causes and results of AS and CR.

Figure 6: Effects of Cardiovascular Risk Factors on Arterial Stiffness and Capillary Rarefaction



Different effects of cardiovascular risk factors on micro- and macrovasculature can increase AS and CR. Increased AS and CR may then mutually deteriorate each other.

AGE= Advanced Glycation End products

ECM= Extracellular Matrix

VSMC= Vascular Smooth Muscle Cell

1.4.3.1 Informative Value of Arterial Stiffness

AS, measured in PWV and aortic AIx, provides information about the function and wall structure of the arteries. These properties do not alter quickly, therefore the measurement of AS provides long-term information about the vascular system rather than a short-term parameter like BP, which can vary from reading to reading.

Processes that decrease arterial elasticity like fibrosis, calcification, and formation of arteriosclerotic plaques are reflected by an increased AS. High PWV therefore implicates an increased risk for cardiovascular events such as myocardial infarction, stroke, and peripheral vascular disease (PVD). The measurement of PWV and AIx can identify these damages already at a subclinical stage of disease [107]. Therefore the measurement of AS can help to target medicinal treatment for asymptomatic patients at risk of CV events. Furthermore, it has been shown that the combination of AS with classical CV risk scores improves the CV risk prediction [108]. The present study investigates the potential benefit of combined assessment of CV risk through classical risk factors, the established independent risk factor AS, and the new biomarker CD.

1.4.3.2 Informative Value of Capillary Density

CD at basal state (BCD) and under venous congestion (MCD) gives information about the supply of oxygen and nutrients for the tissue. It also indicates whether an organ is already damaged as a consequence of an underlying disease such as HTN. Both, PWV and CD, can be used as screening parameters to identify subclinical structural and functional damage of the vascular system [107]. Furthermore, high PWV and low CD are reversible [109-112], so that these measurements may also help to verify the success of treatment.

Whereas considering AS has already been shown to improve CV risk assessment [22], it has not been investigated so far to which extent the combined observation of macro- and microvasculature helps to predict CV risk in comparison with classical risk scores. This investigation seems especially rewarding because different parts of the vascular system may be affected by CV risk factors in different ways.

2. Hypothesis

Combined measurement of AS and CD will improve assessment of cardiovascular risk beyond that predicted by classical scoring systems.

3. Aim

To test the above hypothesis in patients with HTN with or without additional cardiovascular co-morbidity, in particular:

1. The potential practical value of detecting abnormal imaging of the circulation to predict cardiovascular risk, in particular by assessing discrepant findings, e.g. abnormal imaging with normal classical scoring and normal imaging findings despite abnormal classical cardiovascular risk scoring.

2. The value of imaging more than one instance of the circulation as e.g. people with 'normal capillaries' may have stiff arteries and those with 'normal' arteries may have reduced CD. Thus combined assessment for possible sub-clinical macro- and microvascular abnormalities may improve identification of patients at unexpected increased cardiovascular risk when compared to classical risk factor scoring.

3.1 Objectives

The objectives of this study are

- To assess classical risk factor scoring in outpatients with HTN.
- To use validated arteriographic methods [113] to assess AS in these patients
- To use capillary video microscopy [83] to assess resting and maximal perfused CD in these patients
- To compare results for AS with basal capillary density (BCD) and MCD
- To compare results for AS and for BCD/MCD with classical scoring
- To use multivariate analysis to assess predictors of abnormal AS and CD
- To consider the health economics of applying CD assessment as a routine in clinical practice

4. Subjects

The patients included in this study were aged between 20 and 91 years and recruited in the outpatient clinic for HTN, the vascular clinic and the clinic for diabetic foot of the University Hospital of Coventry and Warwickshire between October 2012 and May 2013. Written informed consent was obtained from all patients before performing the measurements.

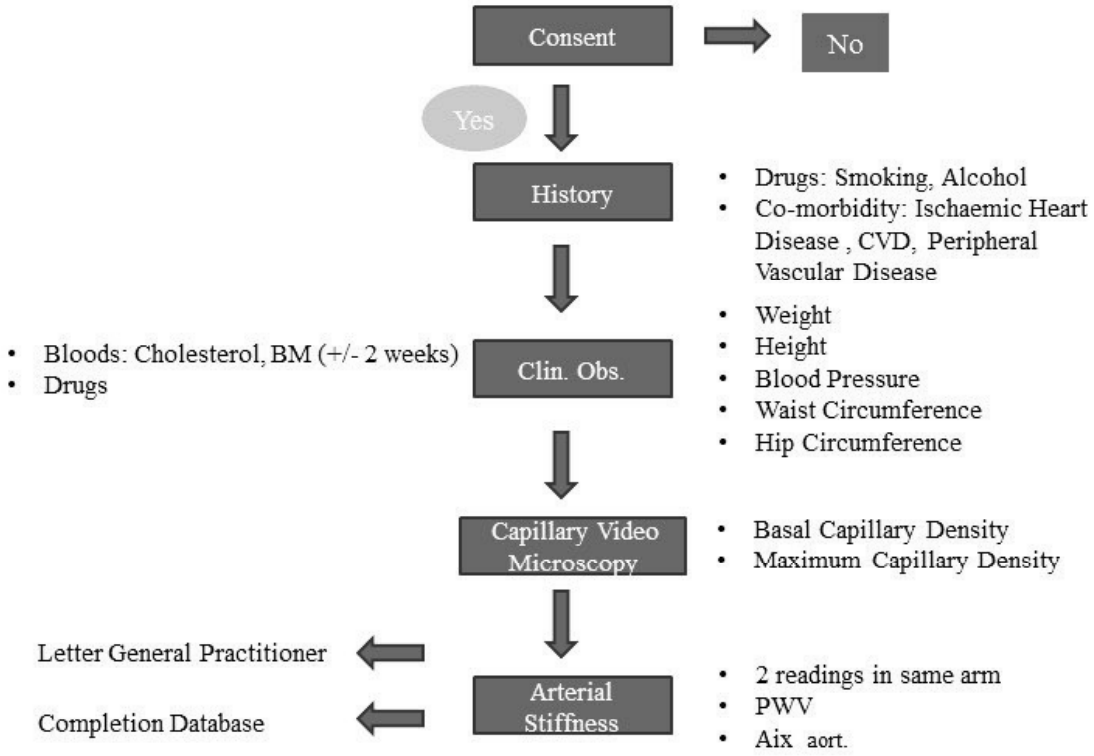
To be included in the study, all patients had to be 18 years or older and have essential HTN, secondary HTN or HTN with cardiovascular co-morbidity. Relevant clinical factors were recorded, including smoking history, lipid status, and diagnosed vascular disease such as ischemic heart disease, cerebro-vascular disease and peripheral arterial disease.

Patients with atrial fibrillation or multiple ectopic beats were excluded from measurements of AS. Capillary video microscopy was not performed in patients with resting tremor, Raynaud´s phenomenon or deep skin pigmentation.

5. Methods

Personal data and measurements in this study were obtained according to the following work-flow.

Figure 7: Work Flow for Cardiovascular Risk Assessment



5.1 Patient Interview

After obtaining informed written consent, the following data was determined.

5.1.1 Personal Information

Gender, ethnicity (Caucasian, Afro-Caribbean, Asian), date of birth and age, smoking history (in pack years) and current smoking status as well as alcohol intake (in units / week) was recorded by standardised interview. One alcoholic unit is equivalent to 10ml of pure alcohol. The recommended alcohol limit for men was 21 units of alcohol per week, no more than four units in any one day and at least two alcohol-free days a week (i.e. 14 units of alcohol per week and no more than 3 units a day for women) [114]. Furthermore, patients were asked about the following pre-existing conditions: HTN, hypercholesterolemia, DM, myocardial infarction / ischemic heart disease, transient ischemic attack / stroke, peripheral vascular disease and CKD. Family history for HTN, DM, myocardial infarction and stroke (before the age of 60) in blood relatives was also obtained.

5.1.2 Examination

The physical examination included the measurement of the patient's height in cm by portable stadiometer with patient wearing shoes, weight in kg by digital scale (accuracy 100g) without heavy clothing, and waist and hip circumference in cm by measuring tape. Waist circumference was taken mid-way between the lowest rib and iliac crest with the patient at the end of normal expiration and wearing thin clothing and hip circumference at the level of the greater trochanters [115]. Mean systolic and diastolic BP was recorded with three consecutive measurements (Omron). The distance between suprasternal notch and pubic bone (sterno-symphyseal distance in cm) was measured in order to calculate the PWV.

5.1.3 Laboratory Data

Creatinine, total cholesterol and HDL, glucose, haemoglobin, and platelet counts were obtained by standard methods.

5.2 Measurements

5.2.1 Capillary Density

Aim of this measurement was to assess capillary function by counting the total number of capillary vessels and the relative perfusion in a representative area of the patient´s finger by capillary video microscopy. This was performed on the dorsum of the middle finger of the non-dominant hand of the patient [84].

5.2.1.1 Different Parts of the Capillary Video Microscope

The capillary video microscope (CapiScope, KK Technology, Devon, UK) consisted of the microscope with a light source, a camera and a brace to fix the patient´s finger and reduce movements during the observation. It was connected to a recorder which saved the videos of the capillaries on tape and a computer which visualised pictures of the dermal capillaries via the included CapiScope software. With this software the tapes were processed subsequent to the measurement.

5.2.1.2 Preparations

Before the beginning of the capillary video microscopy, the patient had to wash the hands thoroughly to remove dust that would disturb the correct assessment of the capillaries otherwise. Then a miniature BP cuff was placed around the root of the middle finger that was used for venous congestion. The finger was placed under the microscope, approximately at heart level, and a small amount of paraffin oil was used to avoid the reflection of the light source at the skin surface.

5.2.1.3 Measurement

The microscope was adjusted manually until the upper layer of the skin with the tips of dermal capillary loops was in focus. CD was measured under resting conditions, showing the functional basic recruitment of dermal capillaries (equivalent to BCD) and under venous congestion, showing the structural maximum number of capillaries (corresponding to MCD).

One field of view was kept in focus for one minute. It was crucial to remind the patient to keep the hand as still as possible. In order to compare the number of capillaries found in different fields of view, this procedure was repeated three times on surrounding spots of the finger dorsum. After completion of this part the cuff was gently inflated above dias-

tolic BP level to cause venous congestion and fill all capillaries of the tissue with erythrocytes. This was done while the fourth field of vision was kept in focus so as to compare the functional and structural CD in the same visual field. After two minutes the maximum number of capillaries was visible under the microscope.

5.2.1.4 Evaluation of the Tapes

To obtain BCD and MCD, all capillaries visible in one field of view during one minute were counted manually, using the counting tool of the Capiscope software. After four different visual fields were recorded under resting conditions, the four readings for BCD were averaged to obtain the mean BCD. MCD was compared to the fourth BCD reading as they were recorded on the same visual field. This was done in order to estimate capillary reserve in the dermal vascular bed, represented by the difference between BCD and MCD.

Figure 8: Capillary Video Microscopy 1: Measurement Tools

Microscope (left) with Capiscope software on screen

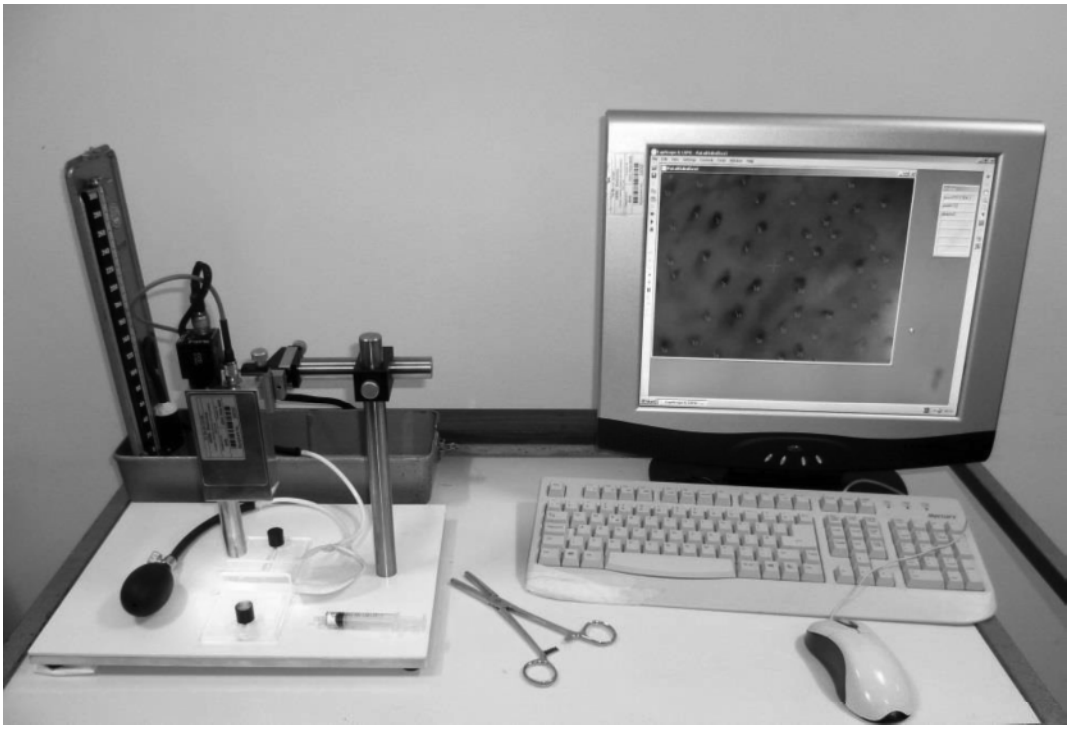
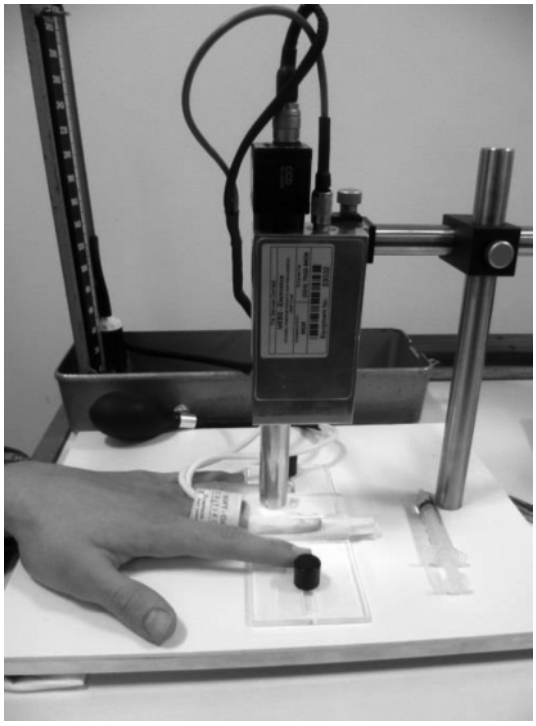
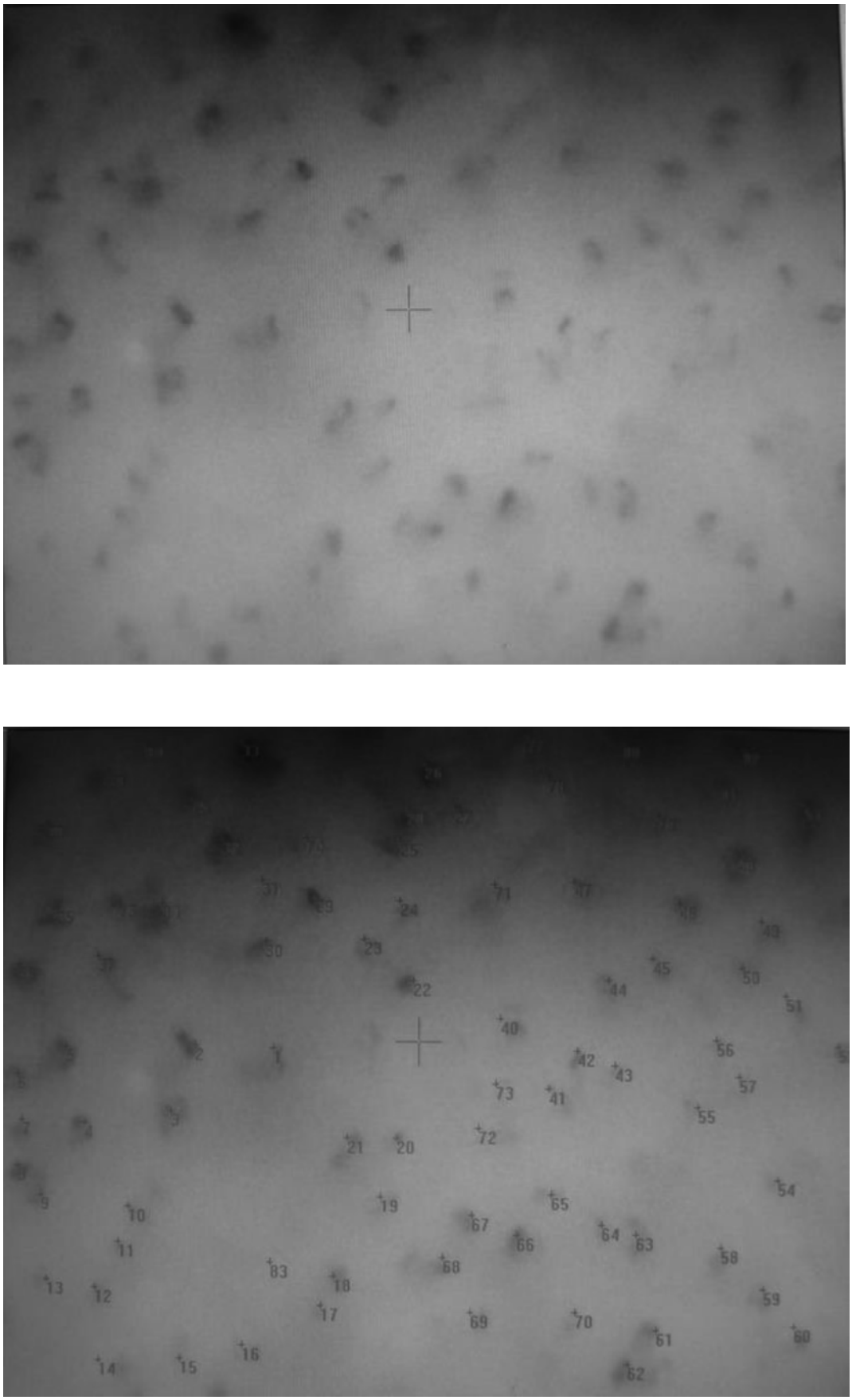


Figure 9: Capillary Video Microscopy 2: Patient Set-Up



Cuff for venous congestion in place, mercury column sphygmomanometer in the background

Figure 10: Basal Capillary Density before (Top) and after Manual Counting (Bottom)



Arterial Stiffness

The AS in this study was determined by the Tensio Clinic arteriograph (TensioMed Ltd., Budapest, Hungary). For validation purposes please see Jatoi et al [113]. The device measured the PWV and the AIx and thus provided information about the functional conditions of the large arteries.

5.2.1.5 Different Parts of the Arteriograph

The device consisted of the arteriograph with a connection to a BP cuff on the one side and an infrared communication window on the other side. This window established an infrared connection via a USB adapter through which the data was transferred to the computer. The TensioMed program installed on the computer then analysed the data and calculated blood pressure, PWV and AIx.

5.2.1.6 Preparations

First the distance between the sternal notch and the pubic bone was measured as it is closely consistent with the length of the aorta from the aortic arch to the point where the pulse wave gets reflected in the femoral artery. This distance was used to calculate the PWV afterwards.

To carry out the measurement the BP cuff belonging to the tool needed to be placed around the upper arm of the patient, an arrow marking the position of the brachial artery. There were three different sizes available. It was crucial to apply the smallest possible cuff as tightly as possible, otherwise the cuff was prone to produce invalid readings. The infrared connection had to be established by placing the infrared window of the arteriograph closely to and at the same height as the infrared adapter of the computer. The patient should not talk or move during the measurement and the cuff should not touch the patient's chest.

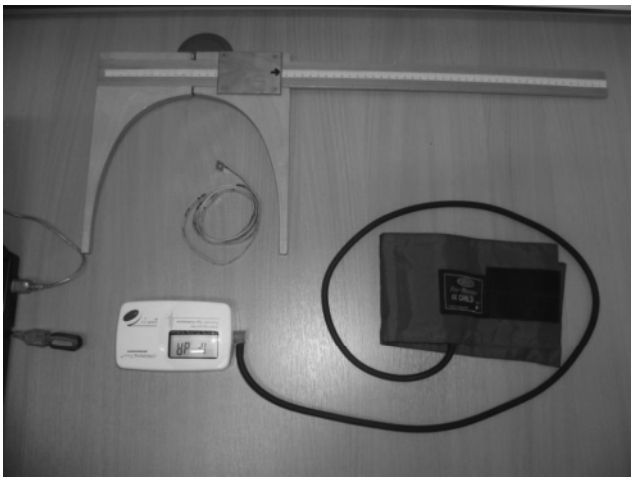
5.2.1.7 The Measurement

Measurement was started by the TensioClinic program. It consisted of three consecutive inflations of the cuff up to different pressure levels. At first the BP was determined; secondly the cuff inflated up to the measured diastolic pressure and thirdly 35 mmHg above the systolic reading. At this last step the patient was told to hold the breath for the length of this phase in order to minimize effects of breathing on the heart rate.

The pulse wave was recorded throughout the measurement and sent via infrared to the computer. To improve the quality of the readings it was aimed to obtain at least two readings for PWV and AIx in each patient.

Figure 11: Arterial stiffness: Measurement Tools and Patient Set-Up

Left: Sliding caliper, tape measure, arteriography with USB flash drive and blood pressure cuff
Right: Set-up for measurement of arterial stiffness: brachial cuff in place, arteriograph with infrared receiver to the left



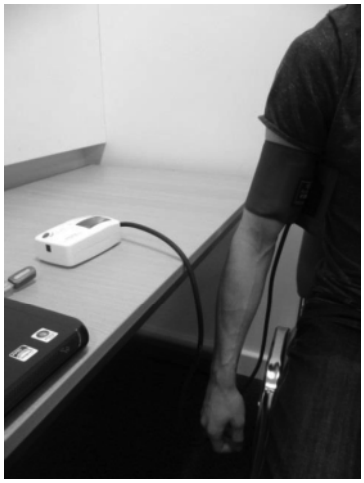
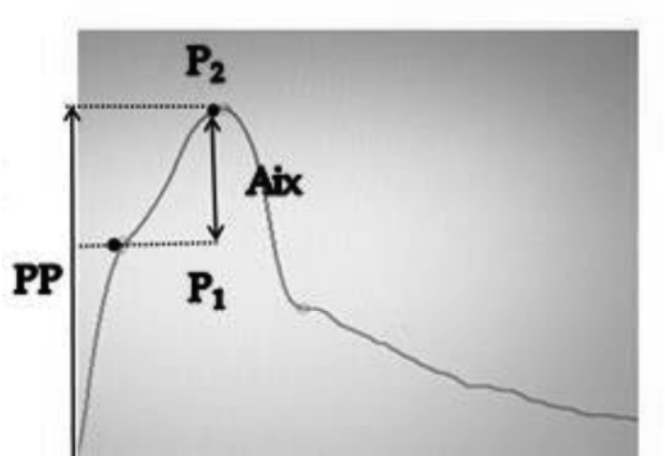


Figure 12: Arterial Pulse Wave Recorded by Arteriograph



PP= Pulse pressure
P_1= Systolic pulse wave
P_2= Reflected pulse wave
Aix= Augmentation Index

5.3 Calculation of Risk Scores

The individual cardiovascular risk of each patient based on classical risk factors was calculated by using two different risk scores: For patients without diagnosed cardiovascular disease, the QRISK2 score was used to determine the 10-year risk for a CV event such as myocardial infarction or stroke [19]. The QRISK2 score was applied because it is based on 16 million patient years of data raised in the UK and Wales and also includes a very detailed selection of classical risk factors as described previously. Furthermore, the wider age range of 25 to 84 years in comparison to other UK risk scores (i.e. JBS2 score with 30 to 74 years [10]) allows the evaluation of a bigger study population. For further information see also www.qrisk.org).

Most common risk scores consider patients with a diagnosis of CVD as high risk in the first place. In order to differentiate further within the group of patients with diagnosed CVD, the Pocock score was used because it also takes into account the cardiovascular history of the individual and calculates the 5-year risk for cardiovascular death [18]. The risk calculator can be accessed under www.riskscore.org.uk.

5.4 Statistics

Data analysis was performed by using IBM SPSS Statistics program version 21. Data was presented as mean ± standard error of the mean (SEM), respectively standard deviation (SD). Bivariate Spearman correlation as well as partial correlation analysis were used to determine correlation between classical risk score or classical risk factors and the measurements of AS and CD.

5.5 Ethics

The study protocol was approved by the Coventry Research Ethics Committee on the 11th March 2008, REC reference number 08/H1210/23. All study participants gave written informed consent.

6. Results

6.1 Characteristics of the Study Group

In this study 150 outpatients with treated HTN, of whom 41 were female, were examined. The mean age was 62.6 ± 1.2 years and the mean systolic BP 146/87± 2/1 mmHg. 72 patients had hypercholesterolaemia, 32 DM, 34 chronic kidney disease, 41 ischaemic heart disease, 27 stroke syndromes and further 47 peripheral vascular disease.

At the time of enrollment into the study, 134 patients were receiving antihypertensive drug therapy whereas 16 had no BP treatment. 43 of these 134 patients had a monotherapy (angiotensin converting enzyme inhibitors 16, calcium channel blockers 16, thiazide(like) diuretics 5, angiotensin receptor blockers 3, β-blocker 1, loop-diuretic 1, and α-blocker/central acting agent 1). 91 patients were under therapy with a combination of these drugs.

Of the 150 patients, 103 had a positive family history for HTN, DM or CV events (MI or stroke) in a blood relative before the age of 60. 41 patients had no such family history. In 6 cases the family history was not available.

Table 5: Characteristics of the Study Group (WHR= Waist-Hip-Ratio)

	Age	**BP sys**	**BP dia**	**Pulse**	**BMI**	**WHR**
Mean ± SEM	62.6 ± 1.2	146 ± 2	87 ± 1	76 ± 1	29.8 ± 0.5	0.98 ± 0.01
Minimum	20	87	50	40	17.84	0.79
Maximum	91	293	121	152	57.29	1.21

Table 6: Frequency of Diabetes Mellitus, Hypercholesterolaemia and Cardiovascular Disease

Gender	**Male patients ∑= 109 (% of study population)**	**Female ∑ = 41 (%)**
DM	24 (22)	8 (20)
Hypercholesterolemia	58 (53)	19 (46)
MI/ angina	36 (33)	5 (12)
Stroke/ transient ischemic attac	24 (22)	3 (7)
PVD	42 (39)	5 (12)
CKD	25 (23)	9 (22)

Table 7: Smoking History and Current Alcohol Use (Mean ± SE)

		Patients (%)	**Use**
Smoking	Never	62 (41)	
	Current	34 (23)	46 ± 6 pack years
	Ex	53 (35)	34 ± 4 pack years
Current alcohol drinking			
	No	55 (37)	
	Yes	92 (61)	19 ± 2 Units/ week

Histograms illustrating the distribution of the core variables used in this study can be found at the end of the results section.

6.2 Readings of Arterial Stiffness and Capillary Density

AS was estimated from aortic PWV and aortic AIx. CD was obtained under resting conditions (BCD) and venous congestion (MCD). Readings of PWV ≥ 10 m/s were regarded as abnormal, indicating increased AS. As a positive AIx indicates an increased second peak of the pulse wave, any $AIx_{aortic} > 0$ % was assessed as pathological. Capillary densities ≤ 59 capillaries per 0.6mm^2 field in the basal state, corresponding with BCD, and ≤ 66 capillaries per field under venous congestion, corresponding with MCD, were assessed as abnormal, indicating CR. These thresholds were chosen in accordance to persistent studies using AS [113] and CD [83, 96].

6.2.1 Overall Means of the Study Population

Table 8 displays the overall means for PWV, AIx, BCD and MCD in the study population. The means of all four core variables are in their respective pathological measurement range.

Table 8: Means of Study Population for Core Variables

	PWV	**AIx**	**BCD**	**MCD**
Mean ± SEM	10.7 ± 0.2	29.5 ± 1.3	53 ± 1	61 ± 1
95% CI	10.3 – 11.4	26.5 – 32.4	51– 56	58 - 64
Median	10.5	28.7	54	61
Interquartile Range	2.9	24.1	14	18
Minimum	4.7	0.2	18	20
Maximum	17.7	59.8	98	104

Readings for PWV and AIx were obtained in 118 patients (78%) and for 124 patients in BCD (82%), respectively. Results were obtained both for PWV (10.8 ± 0.3 m/sec) and CD (BCD 54 ± 1 / field; MCD 62 ± 1/ field) in 100 patients (67%: 95% CI 95 - 105), for PWV alone (10.6 ± 0.63 m/sec) in 18 patients (12%: 95% CI 4 - 32), for CD alone (BCD 50 ± 2/field; MCD 58 ± 2/ field) in 24 patients (16%: 95% CI 11 - 37); and no measurement in 8 patients (3%: 95% CI 0 - 19).

Both PWV and CD were abnormal in 42 patients (42 %: 95%CI 30 - 54). CD was low, but PWV normal in 32 patients (32 %: 95%CI 18 - 46). PWV was raised but CD normal

in 18 patients (18%: 95% CI 11-30); both readings were normal in 7 patients (7% 95% CI 1-13).

Table 9: Means ± SE of Core Variables for Patients with Measurements for Stiffness and/ or Capillaries

Mean ± SE	**PWV**	**AIx**	**BCD**	**MCD**
Both (100)	10.8 ± 0.3	29.3 ± 1.5	54 ± 1	62 ± 1
AS only (18)	10.6 ± 0.6	31.9 ± 3.5		
CD only (24)			50 ± 2	58 ± 2

6.2.2 Readings of Arterial Stiffness and Capillary Density in Different Cardiovascular Risk Groups

Table 10 displays the number and percentage of pathological readings for AS and CD in the different CV risk groups.

Table 10: Pathological Readings for Stiffness and Capillaries in Different Cardiovascular Risk Groups

No. of Patients	**High PWV**	**Low BCD**	**Low MCD**
QRISK low (52)	21 (40%)	38 (73%)	32 (61%)
QRISK high (16)	6 (38%)	9 (56%)	4 (25%)
Pocock (82)	44 (54%)	50 (61%)	46 (56%)

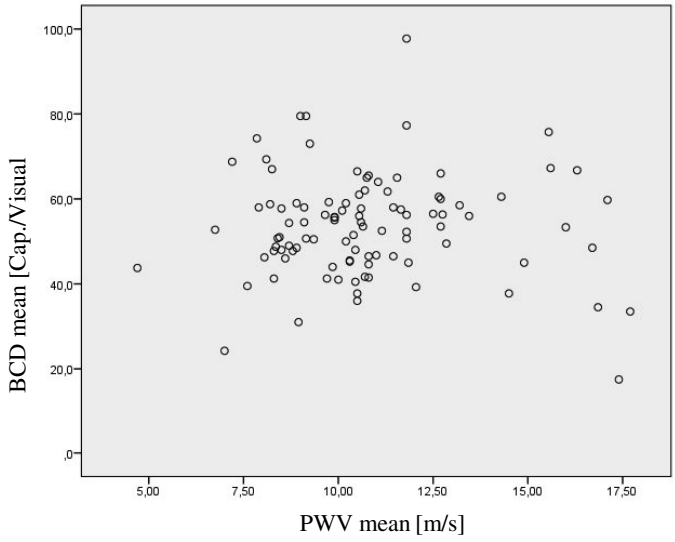
Of particular interest was the finding that 73% of the patients in the "QRISK low" group, regarded as cases of low CV risk, showed an abnormally low BCD and 61% showed low MCD, respectively. Also, 40% of this subgroup displayed a pathologically high PWV. Also in the Pocock group, more than 50% of measurements of PWV, BCD and MCD were pathological.

6.3 Correlations

6.3.1 Arterial Stiffness and Capillary Density

The bivariate analysis of AS and CD showed no significant correlation ($r = -0.034$, $p = 0.740$; $r_s = 0.061$, $p = 0.552$) as illustrated by figure 13.

Figure 13: Univariate Correlation of Basal Capillary Density and Pulse Wave Velocity



6.3.2 Arterial Stiffness / Capillary Density and Risk Scores

AS and CD were subsequently tested for their correlation with the risk scores used in this study. The QRISK2 risk score showed a highly significant correlation with PWV ($r_s = 0.443$; $p = 0.001$), whereas the Pocock score correlated inversely with BCD ($r_s = -0.253$; $p = 0.039$). Log transformation of the right skewed QRISK2 score still showed a highly significant correlation with PWV ($r = 0.452$, $p < 0.001$).

After adjustment for factors that were not included in the risk score itself, such as alcohol intake, kidney function and Waist-hip ratio, the QRISK2 score correlated significantly with PWV, AIx, BCD and MCD, as shown in Table 11.

There were no new significant correlation obtained by adjusting the Pocock score for BMI, alcohol intake and Waist-hip ratio; however, the previous correlation between Pocock risk score and BCD improved slightly ($r = -0.332$; $p = 0.03$), also shown it Table 11.

Table 11: Partial Correlation between QRISK2 and Pocock Risk Score and Core Variables (95%CI)

	QRISK2 r	QRISK p	Pocock r	Pocock p
PWV	0.570 (0.298 – 0.756)	<0.001	n.s.	n.s.
AIx	0.573 (0.302 – 0.756)	<0.001	n.s.	n.s.
BCD	0.325 (-0.003 – 0.59)	0.046	-0.332(-0,58 – 0,028)	0.03
MCD	0.374 (0.052 – 0.625)	0.021	n.s.	n.s.

6.3.3 Arterial Stiffness or Capillary Density and Classical Risk Factors

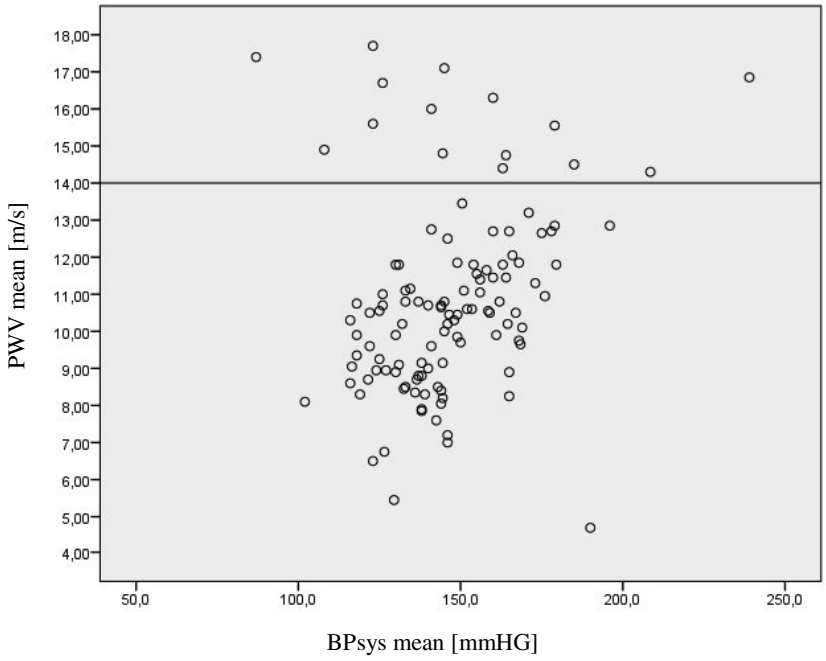
As a first step, Pearson correlation between AS or CD and classical CV risk factors was assessed. Bivariate analysis showed a highly significant correlation of systolic BP and PWV as well as AIx. PWV also correlated highly significantly with pack-years and age. Of note, systolic BP also showed a significant correlation with MCD and diastolic BP with BCD and MCD. There were no significant correlations for BCD and major CV risk factors.

Table 12: Bivariate Correlation of Mayor Cardiovascular Risk Factors and Core Variables (95%CI)

		r	p	r_s	p
BP_{sys}	PWV	0.270 (0.092 – 0.431)	0.004	0.347	<0.001
	AIx	0.373 (0.204 – 0.52)	<0.001	0.358	<0.001
	MCD	0.208 (0.026 – 0.376)	0.026	0.258	0.003
Pack years	PWV	0.242 (0.065 – 0.405)	0.008	0.228	0.001
Age	PWV	0.335 (165 – 0.486)	<0.001	0.361	<0.001
BP_{dia}	BCD	0.186 (0.005 – 0.355)	0.044	n.s.	
	MCD	0.211 (0.031 – 0.378)	0.030	0.210	0.024

By visualising the correlation of PWV and systolic BP, a change of distribution for very high PWV readings became obvious as shown by figure 14.

Figure 14: Distribution of Pulse Wave Velocity in Relation to Systolic Blood Pressure



The plot shows that for very high BP readings, the positive correlation between PWV and systolic BP changes. The subsequent division into subgroups according to PWV showed that the patients with PWV > 13.9 m/s had the highest means for pack years, age and systolic blood pressure.

6.3.4 Partial Adjustment of Correlation

In order to allow for the possible implicit effect of one CV risk factor on the other, each of the major CV risk factors (as represented by systolic blood pressure, pack-years, cholesterol, glucose and creatinine) was analysed in its correlation to AS and CD when adjusted for the remaining four risk factors.

Table 13: Partial Correlation of Mayor Cardiovascular Risk Factors and Core Variables (95%CI)

		r	p
BP_{sys}	PWV	0.369 (0.144 – 0.588)	0.002
	AIx	0.383 (0.16 – 0.618)	0.001
Pack Years	PWV	0.352 (0.138 – 0.534)	0.002
Glucose	PWV	0.304 (0.058 – 0.495)	0.007

Table 14 shows that the correlation of PWV with pack years and systolic BP is preserved also after adjustment for other mayor CV risk factors. Additionally, there is a correlation between PWV and blood glucose levels. There was no significant correlation of BCD or MCD with any of these risk factors, i.e. the previous bivariate correlation between MCD and systolic BP was lost.
There was neither a positive correlation found between BCD or MCD and diastolic BP after partial adjustment for systolic blood pressure.

6.4 Multiple Linear Regression

The following variables entered into the multiple linear regression estimates for PWV: age, pack-years and systolic BP.

f (PWV)= 3.721 + 0.43 x (10 years) + 0.18 (10 pack years) + 0.27 x (10 mmHg)

Table 14: Multiple Linear Regression for Pulse Wave Velocity (1)

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
	0.447[a]	0.200	0.178	2.30807

a. Predictors: (Constant), systolic BP, pack years, age

Table 15: Multiple Linear Regression for Pulse Wave Velocity (2)

ANOVA[a]					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	147.380	3	49.127	9.222	0.000[b]
Residual	591.319	111	5.327		
Total	738.699	114			

a. Dependent Variable: PWV_{mean}

b. Predictors: (Constant), systolic BP, pack years, age

Table 16: Multiple Linear Regression for Pulse Wave Velocity (3)

Coefficients					
Model	Unstandardised Coefficients		Standardised Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	3.721	1.528		2.436	0.016
Age	0.043	0.015	0.251	2.869	0.005
Pack years	0.018	0.008	0.204	2.337	0.021
BP syst.	0.027	0.009	0.265	3.084	0.003

a. Dependent Variable: PVW_{mean}

The statistical power of this model is 0.997, with 3 predictors, observed $R^2 = 0.2$, a sample size of 117 individuals and a probability level of 0.05.

No multiple linear regressions could be modelled for BCD based on the observed parameters.

6.5 Subgroup Analysis

6.5.1 Subgroups for Cardiovascular Risk

6.5.1.1 Characteristics

The study population was split into the following three subgroups according to the individual CV risk: Patients with diagnosed CVD formed the "Pocock group", patients without CVD but a risk ≥ 20% for a CV event during the next ten years formed the group "QRISK high" and the remaining patients without CVD and an intermediate or low CV risk < 20% formed the group "QRISK low".

Figure 15: Flow Chart for Cardiovascular Risk Assessment

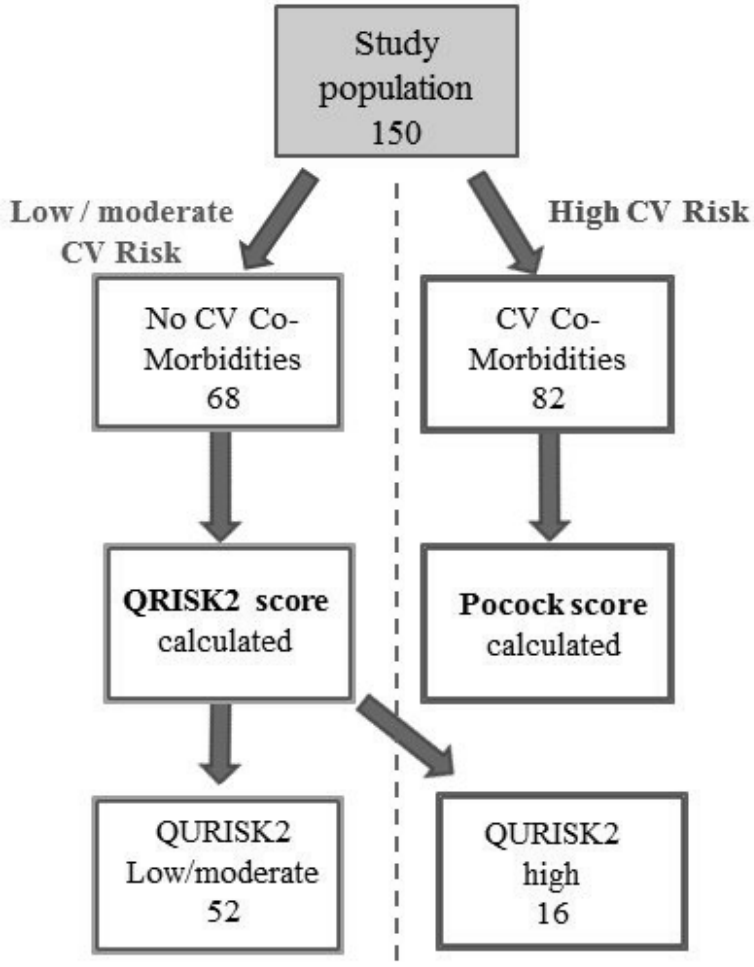


Table 17: Means for Core Variables in Different Cardiovascular Risk Groups

Mean ±SD	**QRISK low n=52**	**QRISK high n=16**	**Pocock n=82**	**ANOVA p-value**
PWV [m/s]	9.9 ±1.7	10.9 ±2.9	11.2 xx ±2.5	0.017
AIx [%]	28.2 ±14.4	37.7 ±14.6	30.4* ±14.3	0.114
BCD [cap./0.6mm^2]	53 ±11	54 ±13	52 ±11	0.176
MCD [cap./0.6mm^2]	61 ±12	67 ±14	61 ±14	0.342
Age [years]	50 ±13	73 ±11	69** xx ±15	<0.001
BP sys [mmHg]	147 ±28	160 ## ±25	145 ±21	0.021
BP dia [mmHg]	92 ±11	90 ±15	84 xx ±14	0.001
BMI [kg/m^2]	31.0 ±3.7	28.6 ±3.5	32.0 ±5.7	0.181
Waist-hip ratio	0.94 ±0.07	0.97 ±0.09	0.99 x ±0.08	0.003
Creatinine [µmol/L]	80 ±19	8 2±22	111 x ±76	0.011
Glucose [mmol/L]	5.1 ±1.3	5.5 ±1.6	5.9 x ±2.0	0.032
Patients with DM	3 (6%)	3 (18%)	26 (31%)	0.001[a]
Cholesterol [mmol/L]	4.9 ±0.8	5.3 ## ±1.0	4.5 x ±0.9	0.001
Patients with Hypercholesterolemia	14 (27%)	7 (44%)	56 (68%)	<0.001[a]
HDL [mmol/L]	1.4 ±0.4	1.5 ±0.4	1.4 ±0.4	0.849
Pack Years	8 ±14	13 ## ±34	30 xx ±31	<0.001
Alcohol [units/ week]	10 ±12	4 # ±16	13 ±18	0.650

Mean ±SD	QRISK low n=52	QRISK high n=16	Pocock n=82	ANOVA p-value
Male Gender	31 (60%)	9 (56%)	69 (85%)	0.002[a]

[a] χ^2-test

Post hoc LSD: QRISK low vs. QRISK high: *=p < 0.05; **= p < 0.001
QRISK low vs. Pocock: x = p < 0.01; xx = p < 0.001
QRISK high vs. Pocock: #= p < 0.05; ##= p < 0.01

6.5.1.2 ANOVA and post-hoc t-test:

There was a significant difference between the means of the three groups for PWV, age, systolic and diastolic PB, waist-hip ratio, creatinine, glucose, cholesterol and pack years based on a ANOVA test for independent samples. The post hoc analysis showed the following significant differences between the three subgroups:

QRISK low vs. QRISK high: significant difference for AIx (p = 0.044) and highly significant difference for age (p < 0.001)

QRISK low vs. Pocock: The comparison of the means showed highly significant difference for PWV (p = 0.005), age (p < 0.001), diastolic BP (p < 0.001), waist-hip ratio (p = 0.001), creatinine (p = 0.005), glucose (p = 0.009), cholesterol (p = 0.005) and pack years (p < 0.001).

QRISK high vs. Pocock: There was a significant difference in the mean alcohol units/ week (p = 0.028) and highly significant difference for systolic BP (p = 0.007), cholesterol (p = 0.001) and pack years (p = 0.007). Of note, the analysis of the subgroups displayed a strong positive correlation between PWV and age, pack years and systolic BP in the QRISK low group only. There were by far more ex-smokers in every group than current smokers.

Table 18: Number of Current and Ex-Smokers in Different Risk Groups

	ex	current
QRISK low (n=52)	14	7
QRISK high (n=16)	7	1
Pocock (n=82)	31	14

6.5.2 Subgroups According to Obtained Measurements

6.5.2.1 Characteristics

According to the numbers listed in table 9 on page 53, the study population was split into four groups depending on which measurements had been obtained for each individual. For patients in group "AS and CD", both measurements had been obtained, for the groups "just AS" or "just CD" only the respective measurement and in the group "none", neither had been obtained.

6.5.2.2 Findings

Patients in the group with AS readings only showed higher mean values for creatinine (148 µmol/l vs. 94 µmol/l) and lower values for haemoglobin (11.9 g/dl vs. 14.2 g/dl) and platelet counts ($213*10^9$/l vs. $257 *10^9$/l) compared to the other groups. Patients in the group with CD only showed a higher number of pack-years (34 vs. 21), as well as higher BMI (32.7 vs. 29.1).

6.5.3 Subgroups According to Pulse Wave Velocity

6.5.3.1 Characteristics

The study population was sub-divided into five groups according to the speed of the PWV: Group 1 with low PWV≤ 8 m/s, group 2 with borderline elevated PWV between 8 and 10 m/s, group 3 with high PWV > 10 m/s, group 4 with very high PWV > 13.9 m/s and group 5 with missing PWV.
The first group comprised 10 patients (7%), the second 37 patients (25%), the third 56 patients (37%), the fourth 15 patients (10%) and the fifth 32 patients (21%).

6.5.3.2 Findings

In the groups with elevated PWV (> 8 m/s), increasing PWV was accompanied throughout the groups with a rise in age, number of pack years, BP, cholesterol and glucose levels and the percentage of patients with diagnosed CVD per group. However, the group with normal PWV (≤ 8 m/s) did not match these tendencies. The means for age, pack-years and cholesterol in the group with missing PWV lay between the ones for high and very high PWV. In accordance to these findings ANOVA testing showed a significant difference for the means of age ($p = 0.005$), systolic BP ($p = 0.009$) and a borderline significance ($p = 0.051$) for pack-years.

Table 19: Means for Core Variables in Different Pulse Wave Velocity Groups

Mean ± SD PWV [m/s]	**Low <8**	**Borderline 8-10**	**High 10-13.9**	**Very high >13.9**	**Missing**	**ANOVA p-Value**
No. of patients	10	37	56	15	32	
[% of stud.pop.]	[21]	[7]	[25]	[37]	[10]	
Age [years]	54*[1]*[2]*[3]	56 x[1] x[2] xx[3]	64	70	66	0.005
	±18	±15	±14	±9	± 14	
BP sys	142	137 xx[1] x[2]	152	157	140 #[1]#[2]	0.009
[mmHg]	±19	±16	±17	±47	±22	
BP dia	86	84 x[1]	91	86	83 #[1]	
[mmHg]	±12	±12	±13	±19	±14	
BMI [kg/m2]	30.1	29.2	29.3	28.46	31.8	
	±6.7	±4.3	±6.1	±5.2	±7.1	
Waist-hip ratio	0.92*[2]*[3]	0.97	0.97	1.0	0.98	
	±0.08	±0.08	0.08±	0.0.8±	0.07±	
Creatinine	109	89	112	97	94	
[µmol/L]	±49	±22	±115	38±	±27	
Glucose	5.7	5.4	5.7	6.2	5.5	
[mmol/L]	±1.8	±1.5	±2.4	±2.3	±1.2	
Cholesterol	4.6	4.5	4.6	4.8	4.8	
[mmol/L]	±0.5	±0.8	±1.0	±0.8	±1.2	
Hemoglobin	13.7	13.8	13.8	13.9	14.4	
[g/dl]	±1.2	±2.5	±1.8	±2.9	±1.8	
Platelets [10^9/L]	240	288	229	250	248	
	±56	±305	±69	±51	±56	
Pack Years	21	12 x[2] x[3]	22	38	29	0.051
	±44	±20	±28	±32	±34	
Alcohol	18	12	12	8	10	
[units/ week]	±19	±17	±20	±13	±15	
% of pat. with CVD	50	35	57	80	63	

Post hoc LSD: *[1] Low – High $p < 0.05$; *[2] Low – very high $p < 0.05$; *[3] Low – Missing $p < 0.05$

x[1] Borderline – High $p < 0.05$; xx[1] $p < 0.01$; x[2] Borderline – Very High $p < 0.01$

x[3] Borderline – Missing $p < 0.05$; xx[3] Borderline – Missing $p < 0.01$

#[1] Missing – High $p < 0.05$; #[2] Missing – Very High $p < 0.05$

6.5.4 Subgroups According to Capillary Density

6.5.4.1 Characteristics

Using the thresholds of 59 capillaries per 0.6mm2 for BCD and 66 capillaries per 0.6mm2 for MCD, 95 patients (63%) had a low BCD (≤ 59 /mm2), 28 patients (19%) had a normal to high BCD (>59/mm2) and in 27 patients (18%) BCD was missing. Regarding MCD, 82 patients (55%) showed low MCD (≤ 66 /mm2), 38 patients (25%) had high MCD (> 66 /mm2) and in 30 patients (20%) MCD could not be obtained.

6.5.4.2 Findings

In groups with high BCD and MCD the percentage of patients with diagnosed CVD, age, PWV and BP were slightly higher than in groups with pathologically low CD. Also, the means for glucose and cholesterol were slightly lower in the groups with high CD. However, only alcohol units / week and haemoglobin showed significant differences in mean ($p = 0.023$ and $p < 0.001$) under ANOVA.

Table 20: Means for Core Variables in Different Capillary Density Groups

Groups: CD [no./0.6mm^2]	**BCD missing**	**BCD low< 59**	**BCD high> 59**	**MCD missing**	**MCD low< 66**	**MCD high> 66**
Number of patients [% of study population]	27 [18]	95 [63]	28 [19]	30 [20]	82 [55]	38 [25]
Age [years]	63	62	65	63	61	64
BP sys [mmHg]	145	145	148	149	142	150
BP dia [mmHg]	87	86	90	90	85	90
BMI [kg/m2]	29.7	29.7	30.3	30.4	29.9	29.4
Waist-hip ratio	0.97	0.98	0.95	0.96	0.99	0.96
Creatinine [µmol/L]	131	95	91	123	98	89
Glucose [mmol/L]	5.9	5.6	5.5	6.2	5.6	5.3
Cholesterol [mmol/L]	4.5	4.8	4.5	4.8	4.6	4.7
Hemoglobin [g/dl]	12.6	14.3	14.4	13.0	14.2	14.2
Platelets [10^9/L]	225	262	234	233	262	236
Pack years	18	23	26	16	26	21
Alcohol [units/ week]	11	10	20	7	13	13
% of patients with CVD	56	52	64	43	56	61

6.6 Timing and Costs of Capillary Density Measurements in Clinical Settings

So far the measurement of CD was not routinely used in daily clinical practice. This work provided useful information about the handling, timing, reliability and other methodical issues of capillary imaging via capillary video microscopy as well as reading of PWV under clinical conditions.

6.6.1 Timing

The preparation of the finger, involving cleaning, fixation and application of paraffin oil required on average four minutes, the subsequent recording of the videos of the skin capillaries required approximately seven minutes for four visual fields under basal perfusion, inflation of the finger cuff to cause venous congestion and one visual field under venous congestion. The evaluation of one set of four BCDs and one MCD through the CapiScope software required on average 25 minutes as the capillaries were manually counted and a picture of each visual field was saved.

6.6.2 Failure

For 27 patients CD could not be obtained. Capillary video microscopy was not performed in five patients because of essential tremor and in eight patients because of dark skin pigmentation due to technical difficulty in performing capillaroscopy in Afro-Caribbean subjects as described in previous papers [83]. In six cases the quality of the records was poor so that these measurements were excluded from analysis. Finally, technical problems impeded the measurement of CD in eight subjects.
Regarding AS, the majority of patients in whom PWV could not be measured had a high BMI and big arm circumference. Four patients had to be excluded because of atrial fibrillation. The number of cases and percentages for different conditions in which PWV, BCD or MCD were not obtained or available are listed in table 21.

Table 21: Reasons for Failure of Measurements

	Reason	Number of patients	% of study group
PWV/ AIx	AF	4	3
	High BMI/ big arm	20	13
	Others[a]	8	5
BCD / MCD	Tremor	5	3
	Dark skin pigmentation	8	5
	Poor quality	6	4
	Technical issues[b]	8	5

[a]includes 1 patients each with very high blood pressure, low heart rate and muscular arm
[b]includes 3 patients in which microscope was not in use

6.6.3 Costs

Based on the required time and costs for personnel and equipment, the costs per test, i.e. measurement of BCD and MCD in one patient, were derived. The estimated costs for the capillary video microscope, room charge and salary for two laboratory assistants working on a 0.75 basis are shown in table 22, added up to £ 43,000 per year. For each patient, an additional material cost of £ 0.3 for tapes and paraffin oil has to be computed.

Table 22: Costs for Measurement of Capillary Density per Year

Purchase video microscope	25,000
→annual depreciation (i.e. costs per year over 5 years)	5,000
Room charge (including electricity costs)	10,000
Salary for two lab. assistants, 0.75 (including training)	28,000
Costs per year	43,000

The measurement of one set of BCD and MCD and the hitherto manual evaluation of the records added up to 40 minutes per patient. The weekly working time of two laboratory assistants working on a 0.75 basis, corresponding six hours daily for each person, added up to 60 hours per week, i.e. 2880 hours per year, assuming 48 working weeks.

In this one-year period, 4320 measurements of CD could be performed and manually evaluated. Therefore, the costs for one test of BCD and MCD under present conditions amount to £ 10.25 as shown in table 23.

Table 23: Calculation of Cost per Test with Manual Evaluation of Capillary Density

1. Working time per year: 60 hrs. x 48 working weeks x 60 min= 172,800 min
2. Basic costs per year: £ 43,000
   Material costs per patient: £ 0.3
3. Number of examined patients per year with manual evaluation:
   (60 hrs. x 48 work weeks x 60 min.) / 40 min/pat. = 4,320
4. Cost per test per patient with manual evaluation:
   (£ 43,000 / 4,320)+ £ 0.3 = £ 10.25

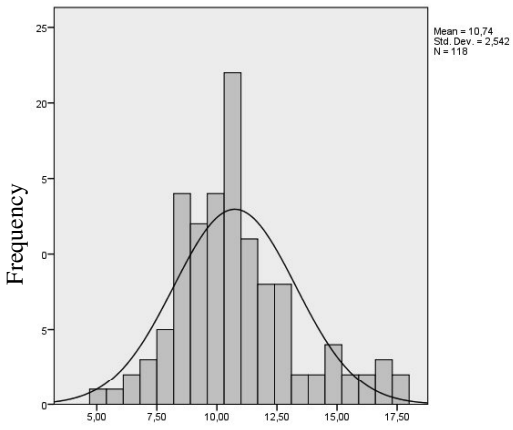
6.6.4 Methodological Issues

Firstly, the correct handling of the capillary video microscope is practice-dependent since the microscope has to be adjusted and maintained in focus manually without changing the visual field. Secondly, blurred or bright pictures prevented the correct assessment of CD because capillaries were hard to identify. Incompliance on the part of the patient caused an unstable visual field. Together, these causes led to the exclusion of the 6 cases mentioned above.

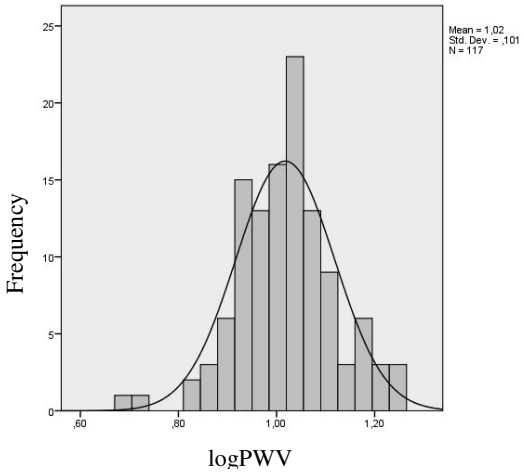
The manual evaluation of the records with 30 minutes per patient to obtain BCD and MCD was time intensive. Therefore, the integration of an autofocus and an automatic counter to evaluate the records would facilitate and speed up the application of the device, improve the quality of the measurements and thus promote its use in clinical practice.

Figure 16: Distribution of Core Variables

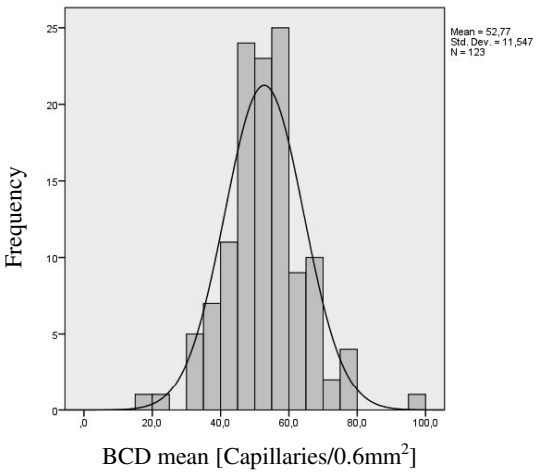
a) PWV



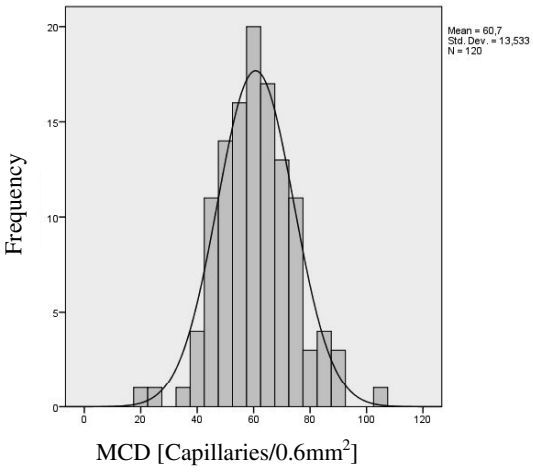
b) log PWV



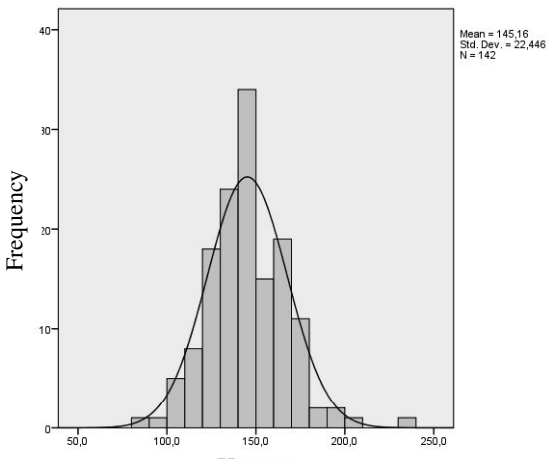
c) BCD

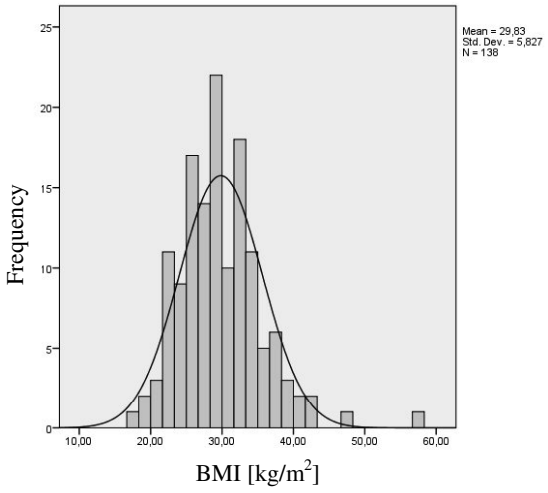


d) MCD

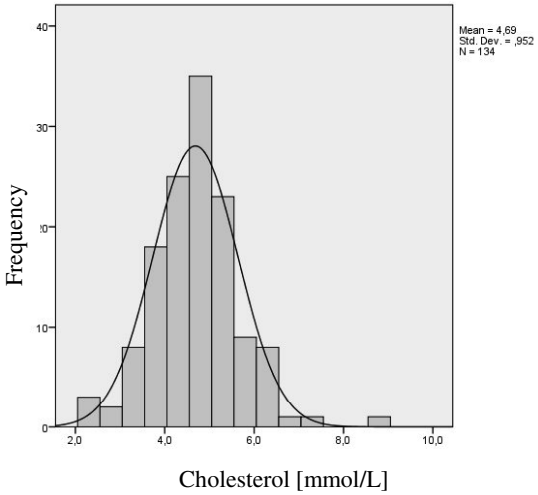


e) Systolic BP





f) BMI



g) Cholesterol

7. Discussion

The aim of this study was to find out whether the measurement of the new microvascular biomarker CD and the established macrovascular biomarker AS would identify patients at unexpected CV risk, as assessed by CV risk scores based on classical CV risk factors, because classical risk factor scores may not always identify CV risk correctly [116,117].

The main findings of this study were that (i) CD was abnormally low in the majority of patients with low CV risk burden based on traditional risk scores; (ii) CD complemented the established large vessel biomarker AS by identifying additional patients with abnormal vasculature and (iii) pathologically low CD and high AS correlated with increased CV risk scoring.
This work is the first to show that CD provides additional value in identifying patients at increased CV risk beyond assessment using classical and non-classical risk factors.

7.1 Capillary Density and Arterial Stiffness in Comparison with Classical Risk Scores

The key question of this research was whether combined measurement of AS and CD would improve assessment of cardiovascular risk beyond that predicted by classical scoring systems. Firstly, more than two thirds of the patients assessed as low to intermediate risk by classical CV risk scores showed pathologically low CD. Over 40% of this group were identified by low CD alone as having an increased CV risk, because these patients had normal PWV and low CV risk scores. This corresponded to 15 % of the study population being at unexpected CV risk, detected by CD alone. Secondly, readings for CD correlated with the Pocock score and PWV with the QURISK score. The inverse correlation of CD and Pocock score (r_s= -0.253, p=0.039) showed that that growing CV risk is accompanied by decreasing CD and was consistent with previous findings of inverse correlation of FRS and CD [112]. The positive correlation of AS and QURISK score (r_s= 0.570, p< 0.001), showing growing AS with increasing CV risk, corresponded to previous findings regarding the relation of AS and CV risk scores such as the FRS [21,118] or other scoring systems [119]. They all demonstrated that AS is a valid biomarker to study CV risk and improves CV risk prediction when added to classical risk scores [108]. Regarding the combined measurement of CD, AS and CV risk scores, only Debbabi et al have performed one study using methods comparable to this study [112]. Although the two studies resemble each other in technical aspects, in Debbabi´s work emphasis was put on the therapeutic effect of HTN control on CD. In contrast, the present work looked particularly into the combined value of low CD and high AS in complementing classical CV risk scores. Neither had the same combination of measurements for CD and AS used frequently in previous studies, nor had the QRISK2 and Pocock

score previously been used in this context.
Therefore, this work is the first to show that quantifiying CD provides additional value in identifying patients at increased CV risk beyond the assessment by classical risk factors and AS.

7.2 Improvement of Risk Assessment By Combination of Vascular Biomarkers

In this study, measurements for micro- and macrovasculature were combined, on the one hand because they provide long-term information about the vascular system for CV risk assessment, on the other hand because people with 'normal capillaries' may have stiff arteries and those with 'normal' arteries may have reduced CD. Up to date, little information is available about the combined assessment of CD and AS, especially when using the precise techniques employed in this study.
In this study population, 74% of the participants showed pathological readings for CD and/or AS. However, large and small vessel readings were only concordant in less than 50% of patients in indicating increased cardiovascular risk. Additionally, no correlation was found between CD and AS. This data is consistent with results by Heitmar et al who examined dermal and retinal capillary function as well as AS in patients with coronary heart disease and did not find any correlation either [120]. Two studies performed by Debabbi et al have assessed AS and CD, focussing on the beneficial effect of BP control on CD [110,112]. Parameters for AS and CD were not compared as in the present study; however, analysis of their data provided for CD and AS neither revealed any significant correlation [112]. Summing up, these discordant findings for AS and CD show that the two measurements seem to identify different patients at increased CV risk. Therefore, CD and AS complement each other in identifying patients at increased CV risk. For this reason both, micro- and microvasculature should be assessed when aiming for CV risk estimation.
Furthermore, these findings could indicate that different pathological effects of CV risk factors may induce the two processes, as described in the background part (p.36 - 38). Hence, the two biomarkers should preferably be evaluated and treated as two separate indicators when aiming for their normalization.

7.3 High Incidence of High Arterial Stiffness in Low-Risk Study Group

Increased AS is now recognised to be of clinical value as an independent cardiovascular risk factor [24]. In this study almost half the study population showed high PWV as a sign of increased CV risk. Already in the sub-group with low CV risk, 40% of the patients had pathological readings of PWV, indicating that increased AS can precede clinical vascular disease in these otherwise healthy individuals. These data are consistent with findings of noteworthy longitudinal studies which have shown an independent risk prediction for CVD events in apparently healthy subjects by increased PWV [21,22]. A highly significant correlation between PWV and systolic BP, age and smoking habit (i.e. pack-years) predominated in the population ($p = 0.004$, 0.008, and < 0.001, respectively), underlined especially by the multiple linear regression for PWV modelled by these three factors. The correlation between PWV and systolic BP was particularly strong in the subgroup with low CV risk burden ($r = 0.744$, $p < 0.001$) which only contained individuals with no known CV co-morbidities. This strong correlation underscored the genuine influence of BP on arterial function. After partial adjustment for other CV risk factors, the correlation with systolic BP and pack-years persisted. Fasting blood glucose levels showed a highly significant correlation with PWV, too. Intriguingly, data analysis showed that the strong positive correlation between systolic BP and PWV seems to decrease for very high PWV-readings, as shown in figure 14 (page 56). As patients with PWV > 13.9 m/s also had the highest means for pack years and age it could be argued that the effect of systolic BP on increasing PWV in an arteriosclerotically altered vascular tree is small, because the arteriosclerotic wall cannot be pre-stretched anymore anyway. Summing up, these results confirmed previous findings that PWV increases with age, BP, diabetes and smoking (pack-years) [48,49,121], illustrating the detrimental effect of these factors on the arterial tree and the reduction of arterial compliance.

7.4 Failure of Arterial Stiffness Measurement as Indicator for High Cardiovascular Risk

In the present study, PWV could not be obtained in 32 cases within a number of maximal three repetitive measurements. Intriguingly, data analysis suggested that the failure of PWV measurement per se may already indicate increased CV risk. Patients of this subgroup were on average older than patients with normal PWV, with higher waist-hip ratio and higher number of pack years. This subgroup also showed a high percentage of patients with CVD and the highest BMI of all groups, although these differences did not reach significance. This high CV risk profile in the group with missing PWV suggested that already the fact of not obtaining a reading for PWV could be a surrogate for increased CV risk. Also, in daily practice increased BMI, i.e. obesity did impede the measurement, as increased arm

circumference hindered the recording of the pulse wave signal by the arteriograph. Taken together, these findings suggest that failure of PWV measurement is an indicator for increased CV risk in itself.

7.5 Capillary Density Identifies Additional Patients at Increased Cardiovascular Risk

Previous studies have shown that CD is low under conditions that increase CV risk such as obesity [89], DM [82] or CKD [103]. In this study group about two thirds of the hypertensive patients displayed low CD under resting condition and/or venous congestion (of all 150 participants, a total of 65% had low BCD and 55% low MCD). Even more, the subgroup with low to intermediate classical CV risk ("QRISK low") presented the highest percentage of pathologically low BCD (73%) and MCD (61%), compared to 56% with low BCD in the "QRISK high" and 61% in the "Pocock" group. Capillary measurement revealed a high incidence of impaired functional and structural microvasculature, especially in patients without major risk for CV events, and thus helped to identify additional patients who might need escalation of their risk factor control. In 12 cases low CD was the only parameter indicating increased CV risk, since PWV was lower than 10 m/s. As the loss of capillaries not only decreases oxygen and nutrient supply within the end organ [81] but also contributes to an increased total peripheral resistance [73], these findings of pronounced CR in low risk patients underscore the idea of CR being a predisposition to acquire further CVD [96,97,104] rather than just a mere consequence of the negative effects of CV risk factors on the vascular bed. If this were the case, the detection of CR in low risk patients were of much higher predictive value regarding than hitherto assumed for the risk of developing CVD. In conclusion, measurement of CD helps to identify additional patients at increased CV risk beyond the assessment by classical risk factors alone. It also shows that CR is already present in individuals with low to moderate CV risk, indicating a possible causative role of CR in the development of future CVD.

7.6 Capillary Rarefaction as an Indicator of Global Cardiovascular Risk

In accordance with previous work [106], this study has shown that CD correlates inversely with CV risk. In order to understand better the possible mechanisms by which CD is affected, BCD and MCD were analysed for correlations with mayor CV risk factors. Further subgroup analysis was done to detect potential differences in CV risk profile within the study group.

Intriguingly, CR did not correlate with one particular risk factor. Considering that patients with a vari-

ety of pre-existing CV conditions, as well as a broad spectrum of drug therapy have been enrolled in this study, many CV risk factors have influenced CD in each individual. However, loss of CD increased with increasing CV risk, especially in the 'Pocock' population, suggesting that the measurement of CD integrates the influences of many adverse factors that act upon the microcirculation. This would support the use of CD as a new biomarker for global CV risk.

Further subgroup analysis showed that the groups with normal CD only differed significantly from the group with pathological CD in their number of alcohol units per week and haemoglobin, however showing higher alcohol consumption and haemoglobin in the group with high CD. Although not on a statistically significant level, age, systolic BP and the percentage of patients with CVD tended to be higher in the groups with normal BCD and MCD, too. This could indicate that the CV risk burden was actually higher in the two groups with normal CD. At this point, it must be taken into account that the study consisted largely of patients under treatment of their risk factors. Hence, patients with a higher risk profile were likely to receive a more aggressive treatment in turn. Therefore, by reverse causation, a more aggressive treatment might have exhibited a positive effect on CD in turn [112].

7.7 Feasibility of Capillary Density Measurement in Daily Practice

In the present study, readings for CD were obtained for 123 hypertensive patients with or without CVD. Over the last twenty years three studies have examined CD in a comparable number of patients fulfilling these criteria [99,110,112]. Two of these studies included smaller numbers of hypertensive patients than this project [99,112]. Furthermore, few information is available regarding the implementation of CD measurement via capillary video microscopy in daily practice. A detailed description of factors such as timing, reliability, methodological issues and costs per test, based on the observations of this study, can be found on page 64 to 66. Under present conditions it took about 40 minutes to obtain readings for BCD and MCD at approximately £ 10 per test.

Time requirements are a main obstacle to the adoption of CD measurements as a routine screening tool in daily outpatient care. Unless automated computerised measurements of BCD and MCD become available and the costs are cut considerably, most practitioners would rather avoid the additional expenses.

Table 24: Estimated Cost per Test with Computerised Evaluation of Capillary Density

1. Estimated number of examined patients per year with computerised measurement: (60 hrs x 48 work weeks x 60 min) / (15 min / patient) = 11,520
2. Estimated cost per patient of computerised CD measurement in a large (prospective) population: (£ 43,000 / 11,520)+ £ 0.3 = £ 4

The coronary heart disease statistics 2012 published data according to which overall CVD is estimated to cost the UK economy £19 billion a year. Of the total cost of CVD to the UK, around 46% is due to direct health care costs, 34% to productivity losses, and 20% to the care of people with CVD [122]. Of the 87.528 deaths caused by CVD in the UK in 2010, 31.645 persons died prematurely, i.e. before the age of 75, causing costs of approximately £ 6.8 billion [122] CD screening might be used in outpatient care to improve risk assessment of known CV patients or to identify patients at increased unexpected CV risk and thus contribute to decreasing adverse outcomes as well as costs for the health care system.

7.8 Inherent Limitations

This project was limited because it only provided a cross-sectional analysis of CD and AS. Therefore its predictive power remains uncertain. As the recruitment of the patients was not confined to treatment-naïve individuals, the possible impact of drugs on CD and AS could not be eliminated.

8. Conclusion and Suggestions for Future Work

As this study collected cross-sectional data, an adequately powered prospective longitudinal study of AS and CD should address the following questions:

- Does CR predict additional adverse CV outcome when compared to standard methods like classical CV risk factors?
- Is low CD in combination with high AS connected with worse CV outcome than low CD plus normal AS?
- How do these biomarkers change under treatment and does a specific treatment to target AS and CD in particular improve CV outcomes?

In the present study capillary imaging identified a quarter of patients at increased risk not detected by classical CV risk factors alone. Large and small vessel readings were only concordant in two out of five patients in indicating increased cardiovascular risk. These findings support the use of diagnostics for both micro- and macrocirculation when assessing clinical cardiovascular risk in patients with HTN. Because of the high incidence of CR in patients with low CV risk profile, measurement of the new microvascular biomarker CD could be a useful addition to classical CV risk assessment, providing functional and structural information about the microcirculation, as well as supporting an earlier detection of a disposition to develop CVD. However, time requirements are a main obstacle to the adoption of CD measurements as a routine screening tool at the moment. Therefore, automated computerised measurements would be necessary for broad adoption in in daily outpatient care

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10. Appendix

10.1 Glossary

ARTERIAL STIFFNESS (AS): An artery's ability to expand during systole and contract during diastole (see also p.24)

CAPILLARY DENSITY (CD): Number of capillaries found per visual field in the capillary bed of a tissue, i.e. capillary loops detected by capillary video microscopy in dermal skin (see also p. 32);

- BASAL CAPILLARY DENSITY (BCD): Number of capillaries per visual field under resting conditions in a tissue (see also p. 43)
- MAXIMUM CAPILLARY DENSITY (MCD): Absolute number of capillaries found per visual field in a tissue, detectable under venous occlusion (see also p. 33)

CAPILLARY RAREFACTION (CR): Phenomenon which describes the decrease of capillaries in the capillary bed of a tissue (see also p. 32 ff.) CR can be quantified by the measurement of capillary density (see above). The term CR can be employed if CD falls under a certain pathological threshold. In this work ≤ 59 capillaries per $0.6mm^2$ field in the basal state, corresponding with BCD, and ≤ 66 capillaries per field under venous congestion, corresponding with MCD were considered as CR.

- FUNCTIONAL CAPILLARY RAREFACTION (FCR): Impaired recruitment of existing, non-perfused capillaries under vasodilating stimulus, i.e. after hypoxia caused by occlusion (see also p. 33)
- STRUCTURAL CAPILLARY RAREFACTION: Anatomical loss and therefore permanent absence of capillaries in the vascular bed (see also p. 33 ff)

10.2 Ethics

NHS

National Research Ethics Service

Coventry Research Ethics Committee
2nd floor West Wing
University Hospital
Clifford Bridge Road
Coventry
CV2 2DX

Telephone: 024 7696 7529
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11 March 2008

Prof D.R.J. Singer
Professor of Clinical Pharmacology & Therapeutics
University Hospital Coventry & Warwickshire NHS Trust
Clinical Sciences Research Institute University Hospital
Clifford Bridge Road Coventry
CV2 2DX

Dear Prof Singer

Full title of study: **New non-invasive, structural and functional biomarkers of cardiovascular and cerebrovascular disease risk**

REC reference number: **08/H1210/23**

Thank you for your letter of 06 March 2008, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chairman.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA. There is no requirement for [other] Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	A&B	28 January 2008
Investigator CV	D R Singer	29 January 2008
Protocol	Version 1	28 January 2008
Covering Letter	Prof Singer	29 January 2008
Peer Review	Dr A Bedlow	29 January 2008

Statistician Comments	Nigel Stallard	28 January 2008
Compensation Arrangements	Zurich Insurance Certificate	01 August 2007
Advertisement	Version 1	29 January 2008
Letter of invitation to participant	Version 1	29 January 2008
GP/Consultant Information Sheets	Version 1	29 January 2008
Participant Information Sheet: Healthy Volunteers	Version 2	06 March 2008
Participant Information Sheet: Patients	Version 2	06 March 2008
Participant Consent Form: Healthy Volunteers	Version 2	06 March 2008
Participant Consent Form: Patients	Version 2	06 March 2008
Response to Request for Further Information	Prof D Singer	06 March 2008

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.
b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.
e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nationalres.org.uk .

08/H1210/23	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Mr Stephen Keay
Chairman

Email: pauline.pittaway@uhcw.nhs.uk

Enclosures: Standard approval conditions SL-AC2

Copy to: Mrs Kate Hughes, University of Warwick

R&D office for UHC&W NHS Trust

10.3 Abstracts

10.3.1 British Microcirculation Society Young Investigators Symposium

April 2013, University of Warwick, UK

COMBINED ASSESSMENT OF CAPILLARY MICROCIRCULATION AND ARTERIAL STIFFNESS TO IDENTIFY CARDIOVASCULAR RISK.

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Abstract

Introduction: Arterial stiffness (AS) and capillary rarefaction are biomarkers of cardiovascular risk.[1] The aim of this study was to assess whether capillary video microscopy may complement measurement of AS to assess cardiovascular risk.

Subjects & Methods: 70 out-patients with treated HTN were studied (age 65.1±1.7(SE)yrs, BP (Omron) 148/88±5/3mmHg, 17F; hypercholesterolemia 40, diabetes mellitus 19, chronic kidney disease 20, myocardial infarction 20, stroke 11, peripheral vascular disease 23). AS was estimated from aortic pulse wave velocity (PWV: Arteriograph, Unimedic); basal (BCD) and maximum skin capillary density (MCD) by video microscopy (KK Technology): abnormal AS PWV≥10 m/s, BCD ≤59 per 0.6mm² field, MCD ≤66/field.

Results: Results were obtained in 48 (69%) patients for PWV (10.9±0.3m/sec) and CD (BCD 50.6±1.7/ field; MCD 60.4±2.0/ field), 10 (14%) PWV alone (11.1±1.0 m/sec), 7 (10%) CD alone (BCD 45.1±2.9/field; MCD 54.0±4.6/ field), 5 (7%) neither measurement. In 24 patients (50%:95%CI 36-64) both AS and CD were abnormal; in 15 (31%:95%CI 18-44) CD low but AS normal; in 6 (13%:95%CI 4-22) AS raised but CD normal; in 3 (6% 95%CI 0-12) both readings normal. PWV correlated with age ($p=0.042$; $r_s=0.298$), systolic pressure ($p=0.009$; $r_s=0.544$) and waist/hip ratio ($p=0.037$; $r_s=0.65$). Aortic AIx (31.4±1.9%) correlated with systolic pressure ($p=0.023$; $r_s=0.483$); and basal with maximum CD ($p<0.001$; $r_s=0.834$). Neither BCD nor MCD were significantly correlated with PWV or AIx.

Conclusions: Large and small vessel readings were only concordant in 50% of patients in indicating increased cardiovascular risk. Capillary imaging identified a third of patients at increased risk not detected by PWV alone. Our findings support diagnostics for both micro- and macrocirculation when assessing clinical cardiovascular risk.

1) Francesco U.S. Mattace-Raso, MD, PhD et al. Arterial Stiffness and Risk of Coronary Heart Disease and Stroke,The Rotterdam Study. Circulation. 2006:113:657-663.

2) Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential HTN. HTN. 1999:33(4):998-1001.

10.3.2 Congress of the International Union of Physiological Sciences

July 2013, Birmingham, UK

COMBINED ASSESSMENT OF CAPILLARY MICROCIRCULATION AND ARTERIAL STIFFNESS TO IDENTIFY CARDIOVASCULAR RISK IN HUMAN HYPERTENSION

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Abstract

Introduction: Both AS (AS) and capillary rarefaction are biomarkers of cardiovascular risk. We assessed whether capillary video microscopy may complement measurement of AS in clinical assessment of cardiovascular risk.

Subjects & Methods: We studied 94 out-patients with treated HTN (age 63.9±1.5(SE)yrs, BP (Omron) 147/87+2/1mmHg, 27F; hypercholesterolaemia 59, diabetes mellitus 23, chronic kidney disease 28, ischaemic heart disease 27, stroke syndromes 16, peripheral vascular disease 31). AS was estimated from aortic pulse wave velocity (PWV: Arteriograph, Unimedic); basal (BCD) and maximum skin capillary density (MCD) by video microscopy (KK Technology): abnormal AS PWV≥10 m/s, BCD ≤59 per 0.6mm2 field, MCD <66/field. Written informed consent was obtained for the studies, which were approved by the local research ethics committee.

Results: Results were obtained both for PWV (11.1+0.2m/sec) and capillary density (BCD 53.4+1.5/ field; MCD 62.1+1.7/ field) in 70 patients (74%: 95%CI 65-83); for PWV alone (10.5+0.8 m/sec) in 8 patients (8%: 95% CI 3-13%); for capillary density alone (BCD 47.6+2.6/field; MCD 59.9+3.4/ field) in 10 patients (11%: 95% CI 5-17); and neither measurement in 6 patients (6%: 95%CI 1-11). Both PWV and capillary density were abnormal in 29 patients (41%: 95%CI 29-53). Capillary density was low but PWV normal in 22 (31%: 95%CI 20-42). PWV was raised but capillary density normal in 14 patients (20%: 95%CI 11-30); both readings were normal in 5 patients (7% 95%CI 1-13). PWV correlated positively with systolic (r_s=0.457, p<0.001) and diastolic BP (r_s=0.265, P=0.021). Aortic AIx (31.9+1.8%) also correlated significantly with systolic (r_s=0.459, p<0.001) and diastolic BP (r_s=0.325, P=0.004). Basal capillary density correlated highly with maximum capillary density (r_s=0.831, p<0.001). There was no significant correlation between PWV and BCD or MCD, before or after adjustment for history of ischaemic heart disease. Waist hip ratio was inversely related to MCD in patients without known ischaemic heart disease (n=57: r_s = -0.264, P=0.047).

Conclusions: Large and small vessel readings were only concordant in 2 out of 5 patients in indicating increased cardiovascular risk. Capillary imaging identified a third of patients at increased cardiovascular risk not detected by PWV alone. Our findings support use of diagnostic tests for both micro- and macrocirculation when assessing clinical cardiovascular risk in patients with HTN with or without cardiovascular co-morbidity.