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Red Cell Distribution Width (RDW) and future risk of arterial cardiovascular diseases

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List of papers

I. Red cell distribution width is associated with incident myocardial infarction in a general population: The Tromsø Study
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II. Red cell distribution width is associated with future risk of incident stroke.
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III. Red cell distribution width and carotid atherosclerosis progression.
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IV. Impact of chronic inflammation, assessed by hs-CRP, on the association between red cell distribution width and arterial cardiovascular disease: The Tromsø Study
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Summary

Cardiovascular disease (CVD) is a collective term comprising all diseases affecting the heart and/or the blood vessels. Atherosclerosis is the primary cause of myocardial infarction (MI) and an important cause of ischemic stroke events. Although the incidence and mortality is decreasing, MI and stroke still rank as the top two killers on the world health organization's causes of death statistics. Understanding and awareness of risk factors is important for targeted prevention of disease, and identification of novel risk factors can further improve the preventive measures. Red cell distribution width (RDW), a measure of the variability in size of the circulating erythrocytes, could be such a risk factor. A growing number of studies are describing a relationship between RDW and arterial CVD. However, most of these studies were limited by their design and thereby unable to conclude on the direction of the observed associations. We therefore aimed to investigate the relationship between RDW and MI, stroke and atherosclerosis in a prospective study of initially disease-free subjects. Further, we wanted to explore the underlying mechanism for the association between RDW and arterial CVD, and especially investigate the impact of chronic inflammation on these associations.

The study population in this thesis was recruited from the fourth (1994-95) and fifth (2001-02) surveys of the Tromsø Study, a large population-based cohort with >27 000 participants. Information on study participants was collected through physical examinations, blood samples and self-administered questionnaires. Atherosclerosis was investigated for, and registered in a standardized way, at both surveys. Incident MI and stroke events during follow-up were registered and validated thoroughly.

In the first paper, we found that RDW was independently associated with incident MI both when modelled as a categorical and a continuous variable. The risk

estimates were independent of anemia and common atherosclerotic risk factors. In the second paper, similar analyses were conducted for incident stroke events. RDW was associated with incident stroke, independent of anemia and atherosclerotic risk factors. The ischemic stroke events were driving this association, with no observed relationship between RDW and hemorrhagic strokes. In the third paper, we investigated the relationship between RDW and growth of atherosclerotic plaques between the fourth and the fifth Tromsø Study survey. RDW was independently associated with growth of atherosclerotic plaques, both in categorical and continuous analyses. The estimates were unaffected by inclusion of high-sensitivity C-reactive protein (hs-CRP) to the multivariable model. In the fourth paper, we investigated the impact of chronic inflammation, assessed by hs-CRP, on the association between RDW, MI and ischemic stroke. Addition of hs-CRP to the multivariable models attenuated the association slightly. Further, we found that RDW was partly mediating the relationship between hs-CRP and ischemic stroke.

Sammendrag

Kardiovaskulær sykdom er et samlebegrep som omfatter alle sykdommer som påvirker hjertet og/eller blodårene. Aterosklerose er den viktigste årsaken til hjerteinfarkt, og en viktig årsak til iskemiske hjerneslag. Selv om forekomsten og dødeligheten faller, er hjerteinfarkt og hjerneslag fortsatt rangert som de to sykdommene som tar flest liv årlig ifølge verdens helseorganisasjons dødsårsak-statistikk. Forståelse av og bevissthet om risikofaktorer er viktig for å kunne utføre målrettet forebygging av sykdommene. Identifisering av nye risikofaktorer kan ytterligere forbedre de forebyggende tiltakene. Red cell distribution width (RDW), et mål på variasjonen i størrelsen til de sirkulerende røde blodcellene, kan være en slik risikofaktor. Et økende antall studier beskriver en assosiasjon mellom RDW og arteriell kardiovaskulær sykdom. Imidlertid er de fleste av disse studiene begrenset gjennom sitt studiedesign, og kan dermed ikke konkludere vedrørende hva som kom først – sykdom eller økt RDW. Vårt mål var å undersøke sammenhengen mellom RDW og hjerteinfarkt, hjerneslag og aterosklerose i en prospektiv studie med initialt sykdomsfrie deltakere. Videre ønsket vi å utforske potensielle underliggende mekanismer for sammenhengen mellom RDW og arteriell kardiovaskulær sykdom, og spesielt undersøke effekten av kronisk inflammasjon.

Studiepopulasjonen i denne avhandlingen ble rekruttert fra den fjerde (1994-95) og femte (2001-02) utgaven av Tromsøundersøkelsen og inkluderte >27 000 deltakere. Informasjon om studiedeltakere ble samlet inn ved hjelp av fysiske undersøkelser, blodprøver og spørreskjemaer. Aterosklerose ble undersøkt etter på en standardisert måte ved både Tromsø 4 og Tromsø 5. Førstegangs hjerteinfarkt og hjerneslag som fant sted i løpet av oppfølgingstiden ble registrert og validert grundig.

I den første artikkelen så vi at RDW var assosiert med førstegangs hjerteinfarkt både når RDW ble modellert som en kategorisk og en kontinuerlig variabel. Risikoestimatene var uavhengige av anemi og vanlige aterosklerotiske risikofaktorer. I andre artikkel ble det utført lignende analyser for tilfeller av førstegangs hjerneslag. RDW var assosiert med hjerneslag, uavhengig av anemi og aterosklerotiske risikofaktorer. Assosiasjonen kunne forklares av de iskemiske hjerneslagene. Vi fant ingen sammenheng mellom RDW og hjerneblødninger. I den tredje artikkelen undersøkte vi forholdet mellom RDW og vekst av aterosklerotiske plakk fra den fjerde til den femte Tromsøundersøkelsen. RDW assosiert med vekst av aterosklerotiske plakk, både i kategoriske og kontinuerlige analyser. Estimatene ble ikke påvirket av høysensitiv C-reaktiv protein (hs-CRP). I den fjerde artikkelen så vi på virkningen av kronisk inflammasjon, målt med hs-CRP, på sammenhengen mellom RDW, hjerteinfarkt og iskemiske hjerneslag. Hs-CRP hadde en svak reduserende effekt på risikoestimatene, men spilte ingen stor rolle. Videre fant vi at RDW delvis medierte forholdet mellom hs-CRP og iskemiske hjerneslag.

Abbreviations

AF	atrial fibrillation
CAD	coronary artery disease
CI	confidence interval
CVD	cardiovascular disease
CHD	coronary heart disease
CRP	C-reactive protein
EPO	erythropoietin
ESR	erythrocyte sedimentation rate
HDL	high-density lipoprotein
Hs-CRP	high sensitivity C-reactive protein
HR	hazard ratio
ICD	international classification of diseases
IHD	ischemic heart disease
IL-6	interleukin-6
IMT	intima media thickness
LDL	low-density lipoprotein
OR	odds ratio
PAD	peripheral arterial disease
PCI	percutaneous coronary intervention
MCV	mean corpuscular volume
MI	myocardial infarction
NLR	neutrophil to lymphocyte ratio
NSTEMI	non-ST elevation myocardial infarction
OxLDL	oxidized low-density lipoprotein
RDW	red blood cell distribution width
RCT	randomized controlled trial
SD	standard deviation
STEMI	ST-elevation myocardial infarction

TPA	total plaque area
UK	United Kingdom
UNN	University hospital of North Norway
US	United States
VCAM	vascular cell adhesion molecule

1. Introduction

Cardiovascular disease (CVD) is a collective term comprising all diseases affecting the heart and/or the blood vessels. In discussion of disease entities related to atherosclerosis, like peripheral arterial disease (PAD), stroke and myocardial infarction (MI), a more narrow term might be used – arterial CVD. This term leaves out diseases related to heart valves, arrhythmias, and the venous system.

The development of an atherosclerotic plaque within the arterial wall is regarded as an inflammatory disease.[1-3] It is the primary cause of thrombotic ischemic stroke, MI and PAD.[4-8] Over the last decades there has been a substantial reduction in both the incidence and mortality rates of MI and stroke.[9-13] This is due to an increased awareness of predisposing factors, improved preventive strategies, and better treatment of acute events.[9, 10, 12] However, heart disease and stroke still rank as the top killers on the world health organization causes of death statistics.[14] In a European update on data from 2012, it was estimated that 46% of all deaths were due to CVD, with coronary heart disease (CHD) accounting for about half of these, and strokes accounting for one fourth.[15] The CVDs account for 37% of the premature (<75 years) deaths in Europe. In the western world, strokes are listed as the third most common cause of death, but ranks highest among causes of permanent disability.[16-18]

Identifying people at risk of disease at an early stage is a fundamental step in the process of decreasing incidence and mortality rates. The arterial CVDs share many modifiable risk factors, including smoking, physical activity, diet, overweight, hyperglycemia, hyperlipidemia and hypertension.[19-28] Awareness of the modifiable risk factors allows for early intervention and prevention of disease. This was recently shown in a Norwegian study reporting a 3% yearly decline in CHD incidence between 1994 and 2010. The study estimated that changes in coronary risk factors accounted for

66% of this decline.[12] In addition to further development of the preventive strategies targeted at the already known risk factors, identification of novel biomarkers is an important step to further decrease CVD morbidity and mortality.

Red blood cell distribution width (RDW), a measure of the variability in size of the circulating red blood cells, might just be such a novel marker. Various CVD outcomes have been related to RDW over the last few years,[29-32] but these studies are all limited by either study design, selected study populations, or issues with reverse causation. Before RDW might be utilized as a marker for disease, the associations must be confirmed in prospective, population-based studies, and the mechanism underlying the associations should be further explored.

There is a great need to identify novel biomarkers for arterial CVD. The relationship between RDW, atherosclerosis, MI and stroke, as well as the impact of inflammation on the relationship between RDW and arterial CVD, will be the topics of the present thesis.

1.1 Erythropoiesis and red blood cell distribution width

The bone marrow of a healthy human produces approximately 10^{12} new red blood cells every day, in a process called erythropoiesis. Through formation and maturation, erythrocytes undergo several phases in which they have different shapes, sizes and names. The more immature a cell is, the larger it is.[33] It all starts with a stem cell, which is transformed into a pronormoblast through steps of different progenitor cells. The pronormoblast is a large cell slightly resembling a red blood cell. The pronormoblast is subdivided further by numerous cell divisions, forming gradually smaller normoblasts. As the cells get smaller, the hemoglobin concentration within each cell increases. Eventually, the nucleus is squeezed out of the cell leaving only some ribosomal RNA capable of hemoglobin synthesis. At this point, the cell is called a reticulocyte, still larger than a mature red blood cell. Normally, the reticulocyte stays in the bone marrow for about 1-2 days before it is released into the blood stream. Once released, the reticulocyte circulates for 1-2 days. During this time, it further shrinks and loses the remaining RNA to form a mature erythrocyte. One pronormoblast normally makes 16 mature red blood cells.[34]

RDW is a measure of the heterogeneity of the circulating red blood cells. It gives the coefficient of variation of the red blood cell volume in percentage, and can be viewed as an electronic equivalent to anisocytosis judged from a peripheral blood smear (Figure 1).[35] The RDW is usually obtained through a traditional full blood cell count by use of automated blood cell counters. This makes it relatively inexpensive and readily available in most clinics. Different hemocytometers use different approaches to calculate the RDW. The most common way, and the one used in all papers of the present thesis, is to calculate RDW by dividing the standard deviation (SD) of the mean corpuscular volume (MCV) with the MCV and multiplying it by 100 to

yield a percentage value. This gives the coefficient of variation of the red blood cell volume around the MCV.[36]

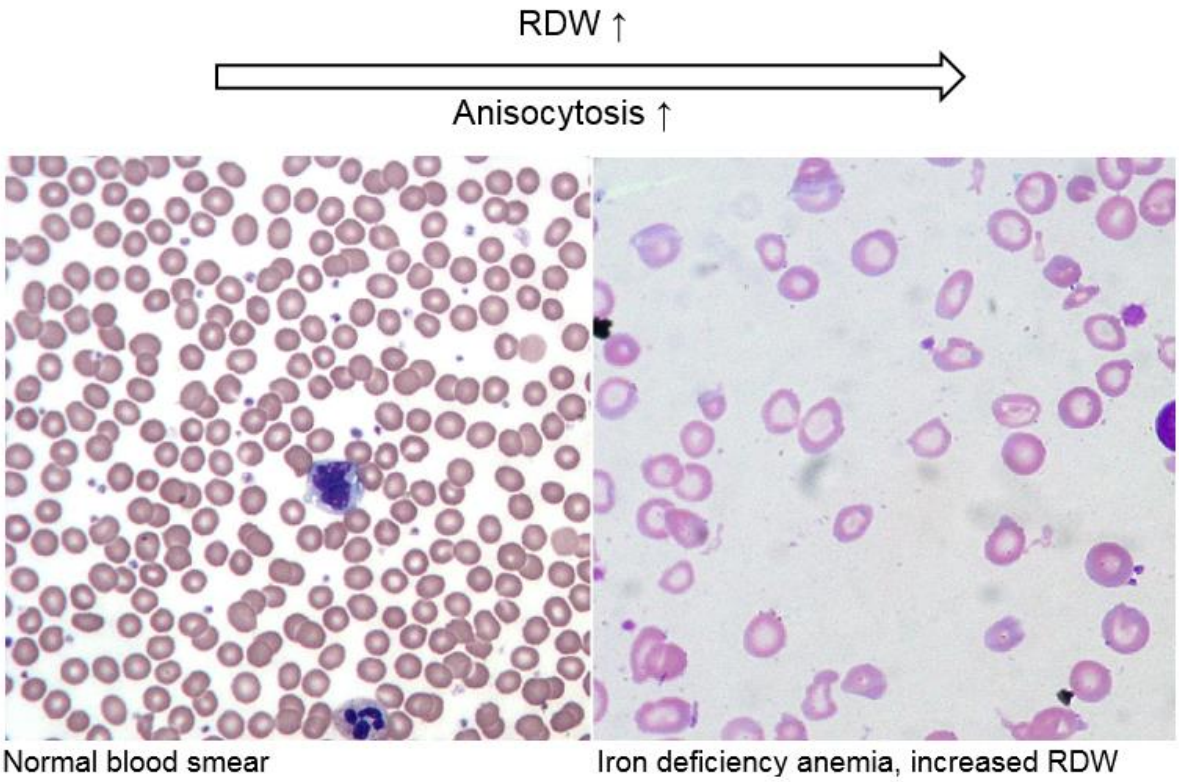

Figure 1. Peripheral blood smear of a healthy individual, and a patient with iron deficiency anemia. This illustrates the difference in the variability of the erythrocyte size. The blood smear from the anemic patient has increased RDW.

Causes of increased RDW include a wide range of diseases that influence the erythropoiesis and alters the release of red blood cells from the bone marrow. Erythropoietin (EPO) is considered the main regulator of the red blood cell production,[37] and plays a crucial role in the final step of erythroid cell maturation.[38] EPO, as well as other factors affecting the erythropoiesis, are important factors in understanding the variation in RDW both intra- and inter-individually. The MCV (and RDW) measurement does not differentiate between mature erythrocytes and circulating reticulocytes. Thereby, release of large and immature erythrocytes

stimulated by an anemic state, may lead to an increased RDW. Traditionally, RDW has been used as a tool in the differential diagnosis of anemia and nutritional deficiencies.[39] B12 and folic acid deficiencies often presents with elevated MCV and RDW, while an iron deficiency anemia will give a low MCV and high RDW. Other hematological conditions such as sickle-cell disease and hemoglobinopathies may also cause alterations of the RDW.[34, 40, 41]

In a study of 26 individuals having monthly blood samples during one calendar year, the intra-individual biological variability in RDW was 3.4%, while the variability due to monthly differences was 1.6%.[42] Studies show that RDW increase with age, while there is no clear evidence of a relationship between sex and RDW.[29, 43]

1.2 Epidemiology

1.2.1 Atherosclerosis in the general population

Atherosclerosis is considered the principal cause of MI and peripheral arterial disease, and an important cause of ischemic stroke.[4-8]

The prevalence of atherosclerosis increases with age. In 526 subjects aged 45-84 years old, there was a rapid increase in asymptomatic atherosclerosis of the carotid artery with increasing age.[44] The prevalence of carotid artery stenosis of any grade was 2.4% among men 55-64 years old. For the men aged 75-84 years, the prevalence of stenosis occluding <50% of the lumen was 30.3%, while 6.1% had stenosis occluding >50% of the lumen. Small lesions, occluding <15% of the lumen, were very prevalent even at relatively young ages, with a prevalence of 32.1% in males aged 45-54 years.[44] A Finnish study evaluated carotid atherosclerosis in males separated in four age groups: 42, 48, 54, and 60 years old.[45] Overall, they found that only 51% were free of any visible atherosclerosis. The prevalence of carotid atherosclerosis increased rapidly across the 6-year intervals, from 14.1% among the 42 year olds, to 32.0%, 67.7% and finally 81.9% among the 60 year olds.[45] They only registered the presence of atherosclerosis, and did not differentiate on magnitude of the disease. A study of transplanted hearts with a mean age of only 33.2 years showed coronary artery lesions in 17% of the hearts.[46] In a study on PAD and claudication, the frequency of intermittent claudication increased across three different age groups. In subjects aged 45-54 years, 55-64 years, and 65-74 years the prevalence of claudication was 0.6%, 2.5% and 8.8%, respectively.[47]

There is a sex difference in atherosclerosis prevalence and morphology, and it changes with age. In a study of 3016 men and 3404 women aged 25-84 years, atherosclerotic plaques were found in 55.4% of the males and in 45.8% of the

females.[48] The turning point for the large sex differences is around 50 years of age, suggesting a menopausal effect on the prevalence among females. Morphology studies showed that the atherosclerotic plaques in males tended to be more prone to rupture, with a more soft and echolucent morphology.[48] In a case-control study of subjects with carotid atherosclerosis, there was a significant linear trend for higher risk of a cerebrovascular event with increasing plaque echolucency.[49]

Atherosclerotic disease seldom affects only one artery or organ. Presence and severity of carotid atherosclerosis is related to both prevalence and extent of CAD.[50-52] A study on renal artery stenosis found that the prevalence of significant disease was higher among subjects with atherosclerosis elsewhere in the body.[53]

1.2.2 Myocardial infarction in the general population

Ischemic heart disease (IHD), a collective term for the consequences of myocardial ischemia, is primarily caused by a MI. IHD is the leading cause of death both worldwide and in Europe.[54, 55] It is estimated that IHD caused 7,249,000 deaths worldwide in 2008, which was 12.7% of the total global mortality that year.[56] In the United Kingdom (UK), the total incidence of MI for people aged 30-69 years, was 6 per 1000 for men, and 2 per 1000 for women in a report from 2007.[57] In an United States (US) update from 2016, the average age-adjusted incidence rate of first MI was 5.3 per 1000 in black men, 3.3 in white men, 3.6 in black women, and 1.9 in white women.[58] In the same study, average age of first MI was 65.1 years for men and 72.0 years for women. Time trends show a decreasing incidence of MI. In a report from the Tromsø population, the age- and sex-adjusted incidence of first ever MI declined by 3% each year from 1994 to 2010.[12] Similar results were reported in a Danish study on 25 year time trends in 234 331 patients with a first time hospitalization for incident MI. From

1984 through 2008, the standardized incidence rate per 100 000 people decreased from 209 to 131 (37%) in women, and from 410 to 213 (48%) in men.[13] According to US data from 2009-2012, the overall prevalence of MI was 2.8% in subjects older than 20 years, 4.0% in men and 1.8% in women.[58] Almost identical numbers were found in the UK, where it is estimated that about 4% of men and 2% of women have had a MI.[57] With over four million deaths yearly in Europe, CVD is responsible for about 51% of deaths in women and 42% of deaths in men, with CHD contributing to about half of these deaths.[15] Although still high, the mortality rates for CHD are decreasing. In the 27 countries of the European Union, CHD mortality decreased by 33% in men and 27% in women from 1985 to 2004.[59] However, the same study showed that in some eastern European countries, the mortality rates increased during the same period. The large Danish nationwide cohort showed a 30-day and 31-365 day mortality decline from 31.4% and 15.6% in 1984-8, to 14.8% and 11.1% in 2004-8, respectively.[13] A study on ethnical differences showed a CVD prevalence of 5.1% in non-Hispanic white men, while 3.6% among non-Hispanic black men, and only 2.6% in Mexican American men.[60] The same study also reported that the incidence of both non-fatal and fatal MI was “delayed” by about 20 years in women compared to men, while the prevalence of age-adjusted angina pectoris was higher among women. In Japan, a country comparable to the western world economically, the rate of CAD is only one fourth of the rate in North America.[60]

1.2.3 Ischemic stroke in the general population

Cerebrovascular events are the primary cause of permanent disability, and the third most common cause of death in the western world.[16, 17, 61] A systematic review of sex differences in stroke epidemiology found a mean age at first-ever stroke of 68.6

years in males and 72.9 years in females. The total stroke incidence was 33% higher in men than in women, but with large variations depending on age and ethnicity.[62] A review of 15 population-based stroke studies found incidence rates ranging from 0.1-0.3 per 1000 person years in subjects <45 years, while for those 75-84 years old the rate was 12.0-20.0 per 1000 person years.[63] Studies on both Japanese and Chinese populations show a higher rate of cerebrovascular events than myocardial infarctions, which is opposite from the situation in western populations.[60] Incidence rates for total stroke range from 1.3 per 1000 person years in the UK, to 4.1 per 1000 person years in Japan.[63] In a study conducted on residents on Manhattan, blacks had a 2.4-fold and Hispanics a 2.0-fold increased total stroke incidence, compared to whites.[64] A similar difference between Mexican Americans and white Americans was reported on ischemic stroke.[65]

The ischemic strokes, which is the main stroke type of focus in this thesis, account for 65-80% of the total stroke events according to a review of population-based studies from around the world.[63] The ischemic strokes are further subdivided in two subtypes, thrombotic and embolic, which have a diverging epidemiology.[66] In a study on ischemic stroke subtypes, the age-standardized incidence rates per 100 000 were 30.2, 25.8, and 15.3 for cardioembolism, small-artery occlusion and large-artery atherosclerosis, respectively.[66] In a follow-up study from the Tromsø population, the age- and sex-adjusted risk of a first-ever ischemic stroke declined by 37.2% from 1995 to 2012.[11] In a study of African Americans, cardioembolic strokes were more than 3 times more common than ischemic strokes due to large vessel disease.[67] A study on Mexican Americans and non-Hispanic whites reported no ethnical difference in the proportion of ischemic stroke subtypes.[68]

In 2006, strokes accounted for about 5.6% of deaths in the US.[69] Between 8-12% of ischemic stroke events are fatal within the first 30 days, among patients aged 45-64 years. A Danish study reported a cumulative risk for death of 28%, 41% and 60% at 28 days, 1 year and 5 years, respectively, after a first ever stroke of any kind.[70] The long-term prognosis was a lot better for the ischemic compared to the hemorrhagic strokes.[70] The same study reported that the increased mortality in subjects suffering a non-fatal stroke, were attributable to concurrent diseases (CVD and cancer), accidents and suicides. The total global, age-standardized mortality rate for ischemic stroke was reduced from 61.3 per 100 000 in 2005, to 48.9 per 100 000 in 2015.[71] Survival has been reported to be poorest among the ischemic strokes with a cardiac source, while the rate of recurrence is higher among strokes due to large vessel atherosclerosis.[72]

1.3 Pathophysiology

1.3.1 Pathophysiology of atherosclerosis

Arteriosclerosis is a collective term for hardening and loss of elasticity of the arterial walls. Atherosclerosis, a type of arteriosclerosis, results from a localized buildup of inflammatory cells, cholesterol and other lipids in the tunica intima zone of arterial walls. This process is termed atherogenesis. Large- to medium-sized elastic and muscular arteries are primarily affected.[1] With time, growing atherosclerotic plaques protrude into the vessel lumen an obstruct blood flow. Normally this takes years and decades to become clinically significant. The atherosclerotic plaques may be harmful either through direct occlusion of the artery at the site of the lesion, or by rupture of the fibrous cap exposing the procoagulant material in the core of the atherosclerotic plaque causing thrombus formation.[73]

Atherogenesis begins with injury to the cells lining the surface of the interior vessel wall, the endothelial cells.[2, 73, 74] The endothelium is recognized not only as a barrier cell, but also a key regulator of vascular homeostasis. It acts as a signal transducer to modify the vessel wall phenotype.[75] Endothelial dysfunction is a consequence of many interfering factors. Such factors include, but are not limited to hypertension, hemodynamics, hyperlipidemia, oxidative stress and inflammation. None of the factors listed are compulsory for disease development, but all increases the risk of endothelial dysfunction. The human arterial system naturally displays the importance of blood pressure in atherosclerosis development. In the pulmonary artery, the average blood pressure is about 25/8 mmHg, which is substantially lower than in the rest of the arterial system. As a result of this, atherosclerosis is more or less absent in the pulmonary circulation, even in subjects with high atherosclerotic burden elsewhere in the body.[76, 77] Atherosclerotic plaques tend to form at points where

arteries branch out, at bifurcations, or at ostia of exiting vessels.[78, 79] The coronary arteries, carotid bifurcation and the infrarenal abdominal aorta are all common sites, due to the disturbed blood flow patterns and high hemodynamic turbulence, which is damaging to the endothelial cells. Common for all risk factors included in atherosclerosis development, is activation of a defensive response in the endothelium. These factors promote atherosclerosis development and growth through increased oxidative stress, as well as activation of molecular processes that result in expression of stimulating cytokines and chemokines. The increase in reactive oxygen species leads to an increased inactivation of nitric oxide, which is an important vasodilator.[80] Reduced vasodilation increases the shear stress on the vessel wall leading to endothelial injury.[81-83] Endothelial injury eventually cause a chronic endothelial dysfunction, and in turn increased permeability through the junctions between the endothelial cells. Increased oxidative stress also causes oxidation of low-density lipoprotein (LDL) contained in the subendothelial space. Oxidized LDL (oxLDL) work as a chemoattractant. It plays a role in adherence of monocytes and T lymphocytes to the endothelium by inducing formation of adhesive cell-surface glycoproteins, like vascular cell adhesion molecule-1 (VCAM-1).[2] When the monocytes and lymphocytes have adhered to the endothelium, oxLDL also affects migration of these cells into the subendothelial space. Once within the intima, monocytes transform into macrophages that devours lipoproteins, like oxLDL. This process further converts the macrophages into so-called foam cells.[84, 85] The activation of these macrophages also leads to cytokine production which recruits additional inflammatory cells and stimulate the adhesion of more monocytes and lymphocytes. T-lymphocytes in the intima interact with macrophages causing increased cytokine cascade activation and production of interleukin-6 (IL-6) and C-reactive protein (CRP). CRP is an acute phase reactant

synthesized and released by the liver in response to signals from macrophages and T-cells.[86] The atherosclerotic plaque is now responsible for a chronic, systemic, inflammatory state.[87, 88] At this point in the atherosclerotic development, the lesion is called a *fatty streak* (Figure 2), and contain mostly monocyte-derived macrophages (foam cells) and T lymphocytes.[89] These lesions are common in young people, and never cause symptoms.[90] In some cases, the fatty streaks develop to atherosclerotic plaques, while in others they disappear completely.[89] The next step on the path to a fully developed atherosclerotic plaque is proliferation and migration of vascular smooth muscle cells and deposition of extracellular matrix, e.g. collagen.[74] This process is stimulated by cytokines and growth factors released by the inflammatory cells present in the lesion.[91] Both the macrophages and the smooth muscle cells are important sources of tissue factor (TF) in the atherosclerotic plaque.[92] TF initiates the extrinsic clotting cascade, and is crucial in both hemostasis and thrombosis.[93] Continued influx of cytokine expressing macrophages, T-cells, mast cells, cholesterol and other lipids will cause the plaque to grow further (Figure 2),[94] with a soft center ultimately degenerating into a necrotic core, and a fibrous cap made out of smooth muscle cells and collagen.

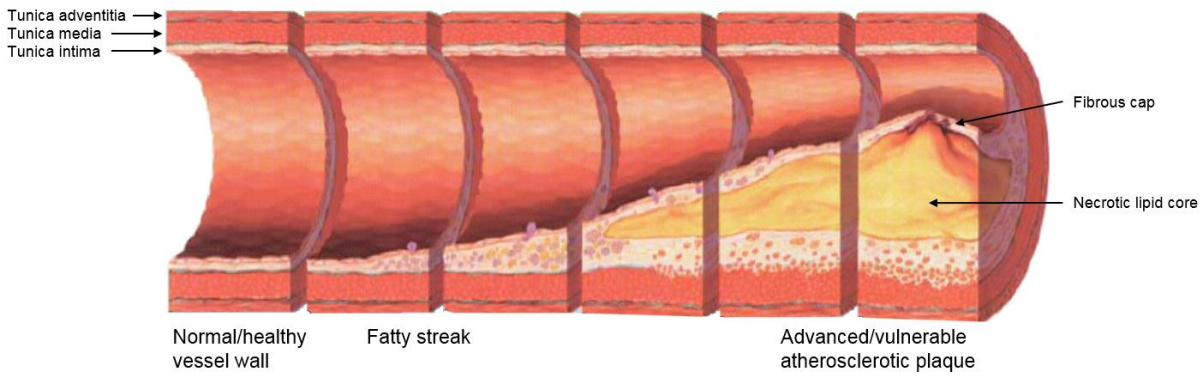

Figure 2. Development of an atherosclerotic plaque. From a fatty streak, a harmless lesion commonly seen at young ages, the vulnerable and dangerous atherosclerotic plaque normally takes years and decades to develop. Adapted from work by Nicholas Patchett (Own work) [CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0)], via Wikimedia Commons.

1.3.2 Pathophysiology of myocardial infarction

When prevention of blood flow and oxygen supply causes ischemia and irreversible damage to the myocardium, it is called a myocardial infarction. For the patient, the MI might present as everything from a small, silent infarction with limited sequela, to a nearly instantaneous deadly outcome. It can be the first sign of CAD (coronary artery disease), or it might occur recurrently in patients with known disease.[95] A MI may result from any cause of increased oxygen demand or reduced oxygen supply. The by far most common cause is a sudden rupture in an atherosclerotic plaque with thrombus formation and coronary occlusion,[96-98] as hypothesized by Herrick more than 100 years ago.[99] Macrophages in the core of atherosclerotic lesions secrete proteolytic enzymes called matrix metalloproteinases (MMPs). The MMPs degenerate the collagen in the plaque, which is responsible for the tensile strength and stability of the fibrous cap (Figure 3).[100, 101] Plaques are under a constant stress by a variety of mechanical and hemodynamic forces. The stress burden is largest in the junction between the plaque and the adjacent healthy vessel wall. The fibrous cap is thinner in this “shoulder” region. Monocytes/macrophages involved in weakening the fibrous cap are mostly situated at the margins of the lipid core, and concentrated in the shoulder area of the plaque.[102] As the site of the largest stress coincides with the site where the fibrous cap is weakest, it is understandable that the shoulder area of the plaque is most vulnerable to disruption.[101] Such a break might be due to a hemorrhage, rupture, ulceration, fissure or some other cause.[103] Once the fibrous cap is broken, the necrotic core rich in TF is exposed to the blood stream. This initiates the coagulation cascade with generation of thrombin. Circulating platelets adhere to the damaged site, aggregate, activate, and release secondary aggregators like thromboxane A_2,

adenosine diphosphate and serotonin.[73] Within minutes, a large thrombus has formed.[74]

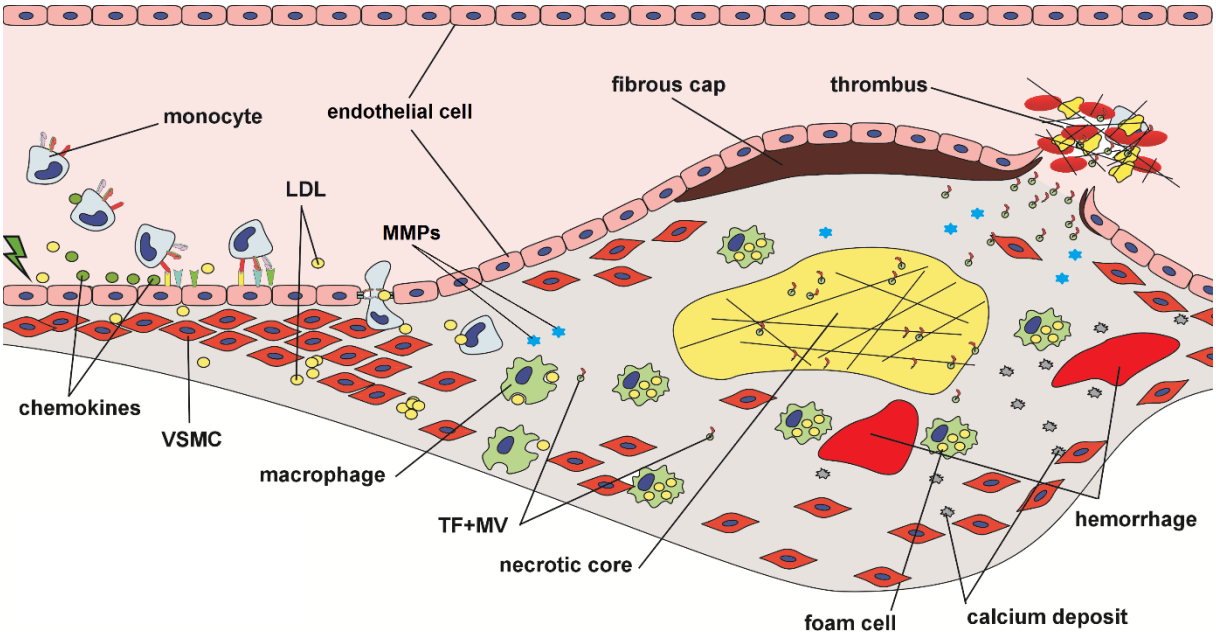

Figure 3.[104] Progression of atherosclerotic lesion with rupture of the fibrous cap and thrombus formation. The initial stages of atherosclerosis are characterized by deposition of lipids in the vascular wall and recruitment of leukocytes. In later stages, formation of necrotic core and foam cells leads to thickening of the vessel wall and consequent rupture of the fibrous cap that ultimately leads to thrombosis. TF+ MV – TF positive microvesicles.

The magnitude of the MI is determined by the duration of the occlusion, size of the area vascularized by the occluded artery, oxygen demand by the myocardium, collateral blood supply, and the severity of the coronary occlusion.[73] It is known that the plaque architecture, biological composition, and blood flow properties are important factors in plaque stability.[105] However, angiographic studies have not been able to classify and determine the type of plaques that are most vulnerable to rupture.[106, 107] Plaque stability is dependent on the composition of the lipid pool, content of inflammatory cells, and the fibrous cap. A large fibrous cap containing a relatively small lipid core is considered a stable plaque. Opposite, plaques with large lipid pools, rich

on inflammatory cells producing degenerating MMPs, and with a thin fibrous cap, are vulnerable to rupture.[3]

1.3.3 Pathophysiology of ischemic stroke

Stroke is defined as a neurologic event caused by death of brain cells due to insufficient blood flow, lasting >24 hours.[108] Stroke is a collective term including two main groups: hemorrhagic and ischemic, with the latter being the focus of this thesis. The ischemic strokes are further subdivided into thrombotic and embolic, with different risk factors, epidemiology and pathophysiology.[109] The thrombotic stroke events have a pathogenesis similar to that of MI, as previously described, with development of atherosclerosis, plaque disruption and subsequent thrombus formation and artery occlusion. The thrombosis might either occlude the artery locally, or travel with the blood stream as a thrombotic embolus.[110] The carotid arteries are common sites of such thrombus formation. Carotid endarterectomy, the surgical procedure of removing atheromatous plaque material from the carotid artery, is highly beneficial in subjects with a cerebral event of expected carotid origin.[111] The cardioembolic stroke events originate from either the atria, ventricles, septum or heart valves, with a pathogenesis unrelated to atherosclerosis. The embolus passes through the left side of the heart into the arterial system and eventually occlude an intracranial artery to cause ischemia. About 50% of the cardioembolic stroke events are caused by atrial fibrillation (AF).[112] Uncoordinated contractions of the atrial appendage with reduced blood flow velocities and stasis is fundamental in the formation of atrial thrombi in AF.[113] The other half of the cardioembolic strokes are caused by a variety of embolic sources including infective and non-infective endocarditis, mitral/aortic stenosis, prosthetic heart valves, myxoma, dilated cardiomyopathy, and others.[114]

1.4 Risk factors for arterial cardiovascular disease

Atherosclerosis, MI and ischemic stroke have many shared risk factors. This is comprehensible as atherosclerosis causes most of the MIs, and many of the ischemic strokes. However, the impact of the common risk factors is different among the three. Due to the nature of the cardioembolic strokes, with a pathogenesis independent of atherosclerosis, other risk factors are more important for these events. Many of the risk factors discussed in the next section also have a complicated interplay. For instance, changes in factors such as physical activity and diet will affect other risk factors like blood sugar, cholesterol and blood pressure.

1.4.1 Modifiable risk factors

Most of the risk factors associated with arterial cardiovascular disease are modifiable through lifestyle changes and/or medication.

Smoking is one example of a well-established and certainly modifiable risk factor. A study of 15152 MI patients from 52 countries found an odds ratio (OR) for MI of 2.87 for current vs never smokers, and a population attributable risk of 35.7% for current/former vs never smokers.[115] In a prospective study of previously healthy female nurses, the number of cigarettes smoked was associated with risk of incident MI. Subjects smoking >25 cigarettes per day had a relative risk of 5.5 for fatal MI and 5.8 for non-fatal MI, compared to non-smokers. Smoking 1-4 cigarettes daily was associated with a 2-fold higher risk of fatal or non-fatal MI.[116] In a Norwegian population, the impact of current smoking on the risk of MI was larger in women (relative risk 3.3), than in men (relative risk 1.9).[117] A meta-analysis on the relation between cigarette smoking and ischemic stroke found a relative risk of 1.9 for smokers compared to non-smokers.[20] A review study on the pathophysiology of cigarette

smoking states that it affects oxidation of LDL, inflammation and thrombus formation.[118] Passive smoking might be hard to modify if living with a smoking spouse or parent. It increases the risk of myocardial infarction, ischemic stroke and cardiovascular mortality.[119-121] A global report on smoking prevalence comparing data from 1980 and 2012 reported a reduction from 41.2% to 31.1% in men, and from 10.6% to 6.2% in women.[122] However, due to the global population growth, the total number of smokers worldwide still increases.

Hypertension, affecting both atherosclerotic plaque formation and rupture, is another very common and potentially manageable risk factor.[123] The prevalence of hypertension among US adults ≥20 years of age was estimated to 32.6% in data from 2009-2012.[58] Hypertension is associated with a 2- to 3-fold increased risk of ischemic stroke and MI.[22] In nine major observational studies with a mean follow-up of 10 years, difference in diastolic blood pressure of 5, 7.5 or 10 mmHg was respectively associated with 34%, 46%, and 56% less stroke events, and 21%, 29%, and 37% less CHD events.[124] The established goal for antihypertensive treatment is a systolic blood pressure <140 and a diastolic blood pressure <90, with a more intensive goal of 130/80 for some high-risk groups.[125, 126]

High cholesterol has been a well-known risk factor for CVD for decades.[26] In six-year follow-up data from the Multiple Risk Factor Intervention Trial, there was a strong association between baseline cholesterol levels and future CHD mortality and death from non-hemorrhagic stroke.[127-129] A beneficial effect of cholesterol lowering drugs on cardiovascular disease outcome have later been shown in several randomized controlled trials (RCT). In 1994, the Scandinavian Simvastatin Survival Study showed that patients with CHD had a clear benefit of treatment with simvastatin, both with regard to recurrent events and mortality.[27] This effect was later confirmed in

studies of different statins.[130-132] The same has been shown for stroke, where simvastatin reduced the risk by 25% in subjects without previous cerebrovascular disease.[133] Primarily due to the introduction of statins in the mid-90s, the prevalence of hypercholesterolemia has fallen. In a US population, the age-adjusted mean total cholesterol concentrations decreased from 5.49 mmol/L and 5.38 mmol/L to 5.16 mmol/L and 5.09 mmol/L in men and women, respectively, between 1980 and 2000-02.[134]

According to a review study, **diabetes** markedly increases risk of MI, stroke and amputation, and most patients with diabetes die from various complications of atherosclerosis.[135] In data from the Framingham study, subjects with diabetes had a 2- to 3-fold increased risk of clinical atherosclerotic disease.[136] Both type 1 and type 2 diabetes are strongly linked to atherosclerosis, MI and stroke.[21, 137-139] In the pathogenesis of CVD, diabetes plays a role in atherogenesis by promoting endothelial dysfunction,[140-142] and in thrombus formation through abnormal platelet activity and coagulation.[143-145] A large study on global trends in diabetes prevalence reported an age-standardized adult prevalence of 9.8% in men and 9.2% in women in 2008. This was an increase from 8.3% and 7.5% in 1980.[146] Obesity is strongly related to the development of insulin resistance and type 2 diabetes.[147] Subjects with a BMI of 40 or higher had an OR of 7.37 (95% CI 6.39-8.50) for diagnosed diabetes compared to normal weight adults.[148]

Patients with **atrial fibrillation** have a five-fold increased risk of stroke,[149] and there is a 2-fold increased risk that a stroke event in AF patients will be fatal.[150] A study on 159 patients with AF experiencing an anterior circulation stroke, found that a cardioembolic etiology was the most likely explanation in 76% of the cases.[151] In a prospective study from the US, AF was associated with a 1.7-fold higher risk of MI.[152]

In a large prospective study of subjects aged ≥55 years, the overall prevalence of AF was 5.5%.[153] The prevalence increased markedly with age, from 0.7% in subjects aged 55-59 years to 17.8% in subjects ≥85 years old. The overall incidence rate was 9.9 per 1000 person-years.[153]

Overweight and obesity markedly increase cardiovascular morbidity and mortality.[25] The development and prevalence of hypertension, diabetes mellitus type 2, and hypercholesterolemia is closely related to overweight and obesity.[154, 155] Interventions to reduce atherosclerotic risk factors in obese individuals lowers the risk of arterial CVD,[156] which further supports the theory that the link between obesity and arterial CVD largely is due to an increase in other atherosclerotic risk factors. The prevalence of obesity (BMI ≥30 kg/m^2) has great variations depending on nationality and ethnicity. In a systematic review of European populations, the prevalence ranged from 4.0% to 28.3% in men and from 6.2% to 36.5% in women depending on nationality.[157] **Physical activity** level is another risk factor closely related to body weight. A large study of European youth demonstrated a clustering of cardiovascular risk factors among subjects with a low average activity level.[158] Habitual physical activity prevents the development of CAD,[159] and exercise level has shown a lowering effect on LDL cholesterol.[160] **Diet** is also linked to body weight and several other cardiovascular risk factors. In a study randomizing participants to either a Mediterranean or a fat-free diet, the Mediterranean diet showed a beneficial change in blood glucose levels, systolic blood pressure, and cholesterol, when compared to a fat-free diet.[161]

1.4.2 Atherosclerosis and risk of MI and ischemic stroke

Ultrasound scanning of the carotid or femoral arteries is a non-invasive way to measure atherosclerotic burden, and several studies have been conducted to investigate whether such measurements can predict arterial cardiovascular events elsewhere in the body.[162-165] The intima and media of both the common and the internal carotid artery was measured in 5858 subjects ≥65 years old. Increased thickness was found to be independently predictive of incident MI and stroke over a median follow-up of 6.2 years.[162] Two systematic reviews and meta-analyses have later confirmed the same, and state that the carotid intima-media thickness (IMT) increasingly is being used as a proxy of coronary atherosclerosis.[163, 164] The relative risk per increase in IMT was slightly higher for stroke than MI.[163] In the Tromsø Study, both carotid IMT and total carotid plaque area predicted first-ever MI, with the latter being the strongest. The relationship was stronger in women than in men.[165] In 2965 subjects followed for an average of 7.2 years, the maximum internal carotid artery added on to the predictive power of the Framingham risk score.[166] Repeated ultrasound-scans of the common carotid artery in 5028 CVD-free subjects, with a median interval of 32 months, showed that IMT progression was predictive of incident stroke.[167] In a study of 391 men with no previous CVD, carotid (OR: 2.09) or femoral (OR: 1.99) atherosclerotic plaques were associated with CVD events independently of other risk factors during 10 years of follow up.[168]

1.4.3 Non-modifiable risk factors

As previously described in the epidemiology section, the risk of arterial CVD is highly affected by age, sex, and ethnicity. Another strong, independent and non-modifiable risk factor for arterial CVD is family history, defined as one or more first-

degree relatives with a history of MI before the age of 60.[169] Several studies have shown that subjects with a parent or sibling with a history of CHD have an increased risk of coronary artery calcification,[170] premature MI,[171-174] and cardiovascular mortality.[175] Having at least one parent with premature cardiovascular disease yielded age-adjusted ORs of 2.0 for men and 1.7 for women.[173] Having a sibling with previous cardiovascular disease yielded an OR of 1.5.[174] Family history of stroke is an independent risk factor for ischemic strokes, but not for the cardioembolic strokes viewed separately.[176, 177] Concomitant familial hypertension and hypercholesterolemia might explain some of the increased risk associated with family history of CVD. Children with familial hypercholesterolemia have an impaired endothelial function, and this impairment is strongest among children with a family history of premature CVD.[178] Interactions have been described between family history of MI and smoking, diabetes, cholesterol levels, and high LDL/HDL ratio.[172, 179]

1.4.4 Inflammation and arterial cardiovascular disease

As previously described, atherosclerosis is recognized as an inflammatory disease. Several studies have described the association between different inflammatory mediators and arterial cardiovascular outcomes. CRP, an acute-phase reactant synthesized in the liver in response to signals from macrophages and T-lymphocytes during inflammation, is a marker of systemic inflammation.[86] In prospective studies on previously healthy men and women, baseline CRP measurements predicted future risk of MI and stroke.[180-183] A meta-analysis of 22 prospective studies on the association between CRP and CHD found an OR of 1.5 for subjects with CRP in the highest tertile compared to the lowest.[181] A review study on various inflammatory markers and atherosclerotic disease reported that population

studies consistently show elevated levels of CRP in subjects that later develop atherosclerosis.[184] CRP adds on to the predictive value of lipid measurements in determining risk of first MI,[185] and is related to future development of PAD.[186]

An inflammatory process always characterizes sites of atherosclerotic plaque rupture, indicating that inflammation plays a role not only in atherogenesis, but also in destabilizing the fibrous cap and thereby enhancing the risk of thrombus formation.[187]

1.4.5 RDW as a risk factor for arterial cardiovascular disease

Over the last decade, some limited scientific evidence supporting an association between RDW and arterial cardiovascular disease outcomes has emerged.

A relationship between RDW and carotid atherosclerosis has been described in patients with hypertension. The cross-sectional study included 156 hypertensive patients aged 60-85 years undergoing carotid ultrasonography with identification of carotid atherosclerotic plaques and measurements of IMT. Both prevalence of carotid plaques and IMT was significantly higher among subjects with higher RDW values.[188] RDW was associated with both presence and complexity of CAD, assessed by the SYNTAX score, in a cross-sectional study of 193 non-anemic patients undergoing coronary angiography for stable angina pectoris.[189] In a cross-sectional study of 6950 non-institutionalized subjects, Zalawadiya et al. found a graded increase in PAD prevalence with increasing RDW quartiles in multivariable adjusted analyses.[190]

Tonelli et al. described the relationship between RDW and MI in a post hoc analysis of 4111 patients with a previous MI. During a median follow-up of 59.7 months, they found a graded, independent relationship between RDW and risk of recurrent non-fatal and fatal MI.[29] Similarly, Lee et al. studied the 12-month risk of a major cardiac

event in patients with a previous MI, and found a graded association between RDW quartiles and the risk of an adverse cardiac outcome.[191] Both these studies are limited by the issue of reverse causation, as they study subjects with a previous MI. In a study of 7556 participants categorized into three groups depending on 10-year Framingham risk of CHD events, each unit increase in RDW increased the odds of being in the intermediate- and high-risk group.[30] Several studies point towards a relationship between RDW and mortality in patients with known CAD. This relationship has been described in patients with non-ST elevation myocardial infarction (NSTEMI) and unstable angina,[192, 193] patients undergoing percutaneous coronary intervention (PCI),[194] and patients with ST-elevation myocardial infarction (STEMI).[195] A few studies have shown contradictory results. In a retrospective study of 225 006 subjects from the Israeli health registry, RDW was associated with an increased risk of all-cause mortality in both sexes, but with cardiovascular morbidity only in women.[31] In a cohort study from Taiwan including 3226 previously healthy subjects followed for 15.8 years, there was no association between RDW quartiles and CAD events.[43]

In a case-control study of 224 incident ischemic stroke events and 224 controls, there was a stepwise increase in the risk of stroke by increasing RDW quartiles.[32] Kaya et al. studied the relationship between RDW and stroke in patients with heart failure. During 1 year of follow-up, there were 14 stroke events among 133 heart failure patients. RDW above 15.2% predicted stroke events with 87% sensitivity and 74% specificity.[196] In subjects with a previous MI, top quartile values of RDW yielded a 2.6-fold higher risk of stroke compared to the bottom quartile.[29]

The current evidence on the relationship between RDW and arterial CVD is limited. A relatively low number of studies have been published on the topic. Further, the published data is limited by selected study populations, study design, and/or

reverse causation. All three studies on RDW and atherosclerosis are cross-sectional, with two of these being small studies on patients with either hypertension or stable angina. The studies on RDW and stroke also have limited numbers, and consist of participants with heart failure or previous MI. Most of the studies on RDW and MI also consist of participants with a previous MI, which gives rise to the issue of reverse causation. The only exception is the prospective cohort study by Chen et al. which describes no association between RDW and CAD events.[43]

2. Aims of the thesis

The overall aims of the thesis were to investigate the association between red cell distribution width and arterial cardiovascular disease, and evaluate the impact of inflammation on this relationship.

The specific aims of the thesis were:

A: To investigate whether red cell distribution width was associated with incident myocardial infarction in a population-based cohort study with validated information on exposure, endpoint, and potential cofounders (Paper I).

B: To investigate whether red cell distribution width was associated with incident stroke in a population-based cohort study with validated information on exposure, endpoint, and potential cofounders (Paper II).

C: To investigate if red cell distribution width was associated with carotid atherosclerosis prevalence and progression in a population-based cohort with validated information on potential confounders (Paper III).

D: To investigate the role of chronic inflammation on the relationship between red cell distribution width and arterial cardiovascular disease (Paper III and IV).

3. Study population and methods

3.1 The Tromsø Study

The Tromsø Study is a single-center, population-based cohort study of the inhabitants in the municipality of Tromsø, Norway. The study was initiated in 1974 with a primary aim to determine causes of the high Norwegian cardiovascular mortality, and to develop preventive methods for myocardial infarction and stroke.[197] Since the beginning, seven surveys have been conducted, with participants undergoing an increasing diversity of investigations allowing for studies on a wide specter of chronic diseases. The fourth survey of the Tromsø Study (Tromsø 4), conducted in 1994-95, invited all inhabitants aged 25 years or older. The study is the largest version to date, with 27 158 individuals attending, yielding a participation rate of 77%. All men aged 55-74 years and women aged 50-74 years, as well as randomly selected 5-8% samples from other five-year intervals <85 years, were offered a more extensive follow-up visit. The second phase visit was completed between 1994-96 with 7965 (76%) attending. The fifth survey of the Tromsø Study (Tromsø 5) was conducted in 2001-02. This survey invited all eligible subjects who had previously taken part in the second phase of Tromsø 4, as well as random samples within different age groups. In total, 8130 (79%) attended Tromsø 5. The participants of Tromsø 5 underwent an extensive screening similar to the one in the second phase of Tromsø 4.

3.2 Study designs

Paper I, II and IV in the thesis were based on data from Tromsø 4. Paper III was based on data from both the Tromsø 4 and Tromsø 5 surveys. In paper I and II, participants were followed from date of enrolment in Tromsø 4 through December 31st 2010. In paper III, participants were followed from enrolment in the second phase of

Tromsø 4 until the extensive screening in Tromsø 5 was completed. In paper IV, the participants were followed from Tromsø 4 until December 31st 2012.

3.3 Baseline measurements

Baseline information was obtained through blood samples, physical examinations, and self-administered questionnaires.

Blood samples were drawn from an antecubital vein into vacutainer tubes containing EDTA as an anticoagulant (K3-EDTA 40 µL, 0.37 mol/L per tube). For preparation of serum, the blood was given a 1 hour respite at room temperature before centrifugation. The blood was analysed at the Department of Clinical Chemistry, University Hospital of North Norway (UNN), Tromsø, Norway. Total serum cholesterol was analyzed by use of an enzymatic colorimetric method with a commercially available kit (CHOD-PAP, Boehringer-Mannheim, Mannheim, Germany). Serum high-density lipoprotein (HDL) cholesterol was measured after precipitation of LDL with heparin and manganese chloride.

For blood cell counts, including RDW, 5 mL of blood was drawn, and analyzed within 12 hours in an automated blood cell counter (Coulter Counter; Coulter Electronics, Luton, UK). The standard deviation of MCV was divided by the MCV and multiplied by 100 to give the RDW. The analytic variation coefficient of RDW was less than 3%.

High sensitivity CRP (Hs-CRP) was measured by a particle-enhanced immunoturbidimetric assay on a Modular P autoanalyzer (Roche/Hitachi) using reagents from Roche Diagnostics GmbH, Mannheim, Germany. The lower detection limit for the assay was 0.03 mg/l, and all measurements below this were set to 0.03

mg/l. Daily changes in the assay precision for values between 0.1 and 20 mg/l was less than 4%.

Trained personnel recorded blood pressure with an automatic device (Dinamap Vital Signs Monitor, 1846, Critikon Inc., Tampa, FL, USA). After two minutes rest in a seated position, three recordings were carried out on the upper right arm with two-minute intervals. The mean of the last two recordings was used in this report. Subjects were defined as hypertensive if they reported current use of blood pressure lowering medication, had systolic blood pressure ≥140mmHg, or diastolic blood pressure ≥90mmHg. Measurements of height and weight were conducted using electronic scales, with participants wearing light clothing and no shoes. Body mass index was calculated as the weight in kilograms divided by the square of height in meters.

Questionnaires were used to obtain information on smoking habits, physical activity, education level, diabetes, cancer, hypertension, MI, stroke, family history of CAD or MI, and medication use including hormone replacement therapy, oral contraceptives, anti-hypertensives and lipid-lowering drugs. The question on diabetes was stated as follows: “do you have or have you had diabetes?” (yes/no). A study participant was defined as a daily smoker if he/she answered yes to any of the following three questions; “do you smoke cigarettes daily?”; “do you smoke cigars/cigarillos daily?”; or “do you smoke pipe daily?”. Study participants answering “no” to all three questions were defined as non-smokers. The questionnaire from Tromsø 4 is attached in the appendix.

3.4 Outcome measurements

3.4.1 Carotid atherosclerosis

Ultrasound scan of the carotid artery was part of the extensive investigation conducted on the study participants attending the second phase of Tromsø 4, and the Tromsø 5 surveys. With the participants in a supine position, high-resolution B-mode and colour Doppler/pulsed-wave Doppler ultrasonography of the right carotid artery was performed from just above the clavicle to as far distal to the bifurcation as possible. All sonographers had completed a two-month pre-study training to ensure an equal and standardized examination technique and measurement procedures. An Acuson Xp10 128 ART ultrasound scanner equipped with a linear array 5-7 MHz linear transducer was used for the examination in both Tromsø 4 and 5. The subjects were randomly distributed among the sonographers, who were blinded to the laboratory data and data from the questionnaires.[198]

Plaques were investigated for in the near and far walls of the right common carotid, the bifurcation, and the internal carotid artery (6 locations). A plaque was defined as localized protrusions of more than 50% compared to adjacent IMT. The examinations and measurements of all plaques were recorded on videotapes. Subsequently, the stored B-mode images were digitized with the use of meteor II/Matrox Intellicam, a commercially available video grabber card. Plaque area was assessed with Adobe Photoshop image-processing program (version 7.0.1), by tracing the plaque perimeter with a cursor.[199] The outline of each plaque was marked manually on still images, with calculation of plaque area. In subjects with more than one plaque, total plaque area (TPA) was calculated as the sum of all plaque areas. Plaque progression was defined as the difference in TPA from Tromsø 4 to Tromsø 5. Reproducibility of the ultrasound examinations was acceptable.[198, 200]

3.4.2 Myocardial infarction

The unique Norwegian national 11-digit identification number allowed linkage to national and local diagnosis registries. All first-time events of MI were identified by linkage to the diagnosis registries at UNN (outpatient diagnoses included) and the National Causes of Death Registry at Statistics Norway. Cases of possible incident nonfatal and fatal MI were identified by a broad search for the International Classification of Diseases (ICD) 9th revision codes 410 to 414, 430 to 438, and 798 to 799 in the period 1994–1998, and thereafter for the ICD 10th revision codes I20 to I25, I60 to I69, and R96, R98, and R99. The Causes of Death Registry covers participants registered as living in Norway at the time of their death, regardless of whether the death took place in Norway or abroad.

An independent endpoint committee validated all possible events of MI. The hospital medical records were retrieved for case validation. Information from the National Causes of Death Registry and from death certificates was used to collect relevant information of the event from additional sources such as autopsy reports and records from nursing homes, ambulance services, and general practitioners. Manual and/or electronic text searches in paper versions (used until 2001) and digital versions of hospital records for notes on MI in all participants with one diagnosis or more of those mentioned above was performed. A systematic text search for MI was also performed in participants with one of the diagnoses other than MI. We included all incident events classified as definite, probable, or possible MI, based on a classification algorithm that included clinical symptoms and signs, findings in electrocardiograms, values of cardiac biomarkers, and autopsy reports, when applicable (Table 1).

Definite MI	Definite MI was defined by one of the following conditions: 1. Typical, atypical, or inadequately described symptoms plus a definite new infarction in ECG recordings 2. Typical symptoms plus significantly higher myocardial enzyme and/or troponin levels 3. Atypical or inadequately described symptoms plus significantly higher myocardial enzyme and/or troponin levels plus a probable new infarction in ECG recordings 4. Postmortem evidence of a recent MI or thrombosis
Probable MI	Probable MI was defined by one of the following sets of conditions: 1. Typical, atypical, or inadequately described symptoms plus probable new infarction shown in ECG recordings plus moderately increased myocardial enzyme and/or troponin levels 2. Typical symptoms plus moderately higher myocardial enzyme and/or troponin levels 3. Atypical or inadequately described symptoms plus significantly higher myocardial enzyme and/or troponin levels 4. Atypical or inadequately described symptoms plus moderately higher myocardial enzyme and/or troponin levels plus probable new infarction shown in ECG 5. Sudden death with no evidence of noncoronary cause of death
Possible MI	An event that can be dated and for which secondary data of typical history in combination with ECG findings and/or echocardiography and/or autopsy are consistent with MI but for which no primary data source is available
Unstable angina	Angina at rest or minimal exertion and ST-depression or negative T-wave in ECG
Unclassifiable	Increase in troponins or enzymes in relation to cardiac revascularization procedures (percutaneous coronary intervention or coronary artery bypass grafting) or otherwise unclassifiable
Silent MI	In the absence of clinical symptoms that can be dated: 1. New diagnostic Q-wave in incidental ECG, or 2. Evidence of MI on echocardiograph and/or multigated acquisition scan, or 3. Evidence of MI at autopsy
No MI	The conclusion after the validation procedure is that the event does not fulfill the criteria for an acute coronary event

Table 1. Classification algorithm for Myocardial Infarction. The Tromsø Study

3.4.3 Stroke

Stroke was defined according to the WHO definition as rapidly developing clinical signs of focal or global disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, and with no apparent cause other than vascular origin.[201] The Norwegian national 11-digit identification number allowed linkage to national and local diagnosis registries. Incident non-fatal and fatal strokes were identified by a search for the ICD 9th Revision codes 430-438 and ICD 10th Revision codes I60-I69 in the diagnosis registries at the UNN (diagnoses from outpatient clinics included), and the National Causes of Death Registry at Statistics Norway. The Causes of Death Registry covers all subjects registered as inhabitants of Norway at the time of their death, regardless of whether the death took place in Norway or abroad. This ensured a complete follow-up status for all-cause mortality.

An independent endpoint committee validated all possible in-hospital and out of hospital stroke events. Hospital medical records were retrieved for case validation. Information from the National Causes of Death Registry and from death certificates was used to collect relevant information of the event from additional sources such as autopsy reports and records from nursing homes, ambulance services, and general practitioners. Event ascertainment followed a detailed protocol, according to established diagnostic criteria. Each case was reviewed separately. Manual and/or electronic text searches in paper versions (used until 2001) and digital versions of hospital records for notes on stroke in all participants with a diagnosis of ICD-9 410-414, 798-799, and ICD-10 I20-I25, R96, R98, and R99 was performed.

4. Main results

4.1 Paper I

RED CELL DISTRIBUTION WIDTH IS ASSOCIATED WITH INCIDENT MYOCARDIAL INFARCTION IN A GENERAL POPULATION: THE TROMSØ STUDY

RDW is associated with cardiovascular morbidity and mortality in selected populations. However, the association between RDW and MI in a general population remains scarcely investigated. Therefore, we studied the relationship between RDW and incident MI in 25612 participants recruited from the Tromsø Study, a large population-based cohort. In Cox-regression models adjusted for common cardiovascular risk factors, we calculated incidence rates and hazard ratios (HR) with 95% confidence intervals (95% CI) for incident MI across quintiles of RDW. During a median follow-up of 15.8 years, 1779 subjects suffered a first-ever MI. A 1% increment in RDW yielded a 13% increased risk of MI (HR: 1.13, 95% CI 1.07-1.19), demonstrating a linear association. In categorical analyses with the lowest RDW quintile as reference, subjects in the highest quintile had a 34% higher risk of MI (HR: 1.34, 95% CI 1.11-1.16), while the risk was 71% higher for those with RDW values above the 95th percentile (HR: 1.71, 95% CI 1.34-2.20). The risk estimates barely changed after exclusion of anemic subjects (n=1297). When stratified according to smoking status, the association between RDW and MI was stronger among current smokers than non-smokers. Smokers with RDW values >95th percentile had a 2.3-fold increased risk of MI compared to smoking subjects in quintile 1 (HR: 2.28, 95% CI 1.55-3.35). In conclusion, RDW was associated with incident MI in a general population independent of anemia and cardiovascular risk factors.

4.2 Paper II

RED CELL DISTRIBUTION WIDTH IS ASSOCIATED WITH FUTURE RISK OF INCIDENT STROKE

The relationship between RDW and incident stroke in a general population remains unknown. We aimed to study if RDW was associated with incident stroke and case fatality in subjects recruited from a large population-based cohort, the Tromsø Study. Study participants were recruited from the fourth survey of the study, conducted in 1994/95, and 25 992 subjects were included. Incident stroke events were registered from enrolment until December 31, 2010. Multivariable Cox regression models were applied to calculate HR with 95% CI for stroke across quintiles of RDW, and per 1% increase in RDW, adjusted for age, sex, BMI, smoking, hemoglobin, white blood cell count, thrombocyte count, hypertension, total cholesterol, triglycerides, self-reported diabetes, and red blood cell count. Through a median follow-up of 15.8 years, there were 1152 incident stroke events. In multivariable analyses, subjects with RDW in the highest quintile had a 37% higher risk of stroke compared to subjects in the lowest quintile (HR: 1.37, 95% CI 1.11-1.69). The risk estimate was further increased when comparing subjects with RDW values above the 95th percentile with those in the lowest quintile, with a HR of 1.55 (95% CI 1.16-2.06). A 1% increment in RDW yielded a HR for stroke of 1.13 (95% CI 1.07-1.20), indicating a clear dose-response relationship. The ischemic stroke events (n=998) were driving most of the association, with subjects in quintile 5 having a 30% higher risk of ischemic stroke compared to those in quntile 1 (HR: 1.30, 95% CI 1.04-1.64). All estimates remained essentially the same after exclusion of 1102 anemic subjects. No association was seen between RDW and case fatality. In conclusion, RDW is associated with incident stroke in a general population, independent of anemia and traditional atherosclerotic risk factors.

4.3 Paper III

RED CELL DISTRIBUTION WIDTH AND CAROTID ATHEROSCLEROSIS PROGRESSION

The relationship between RDW and atherosclerosis is only investigated in cross-sectional studies. We aimed to assess the relationship between RDW and formation and growth of carotid atherosclerotic plaques in subjects recruited from the general population. Baseline measurements, including RDW, were collected from 4677 participants attending the second phase of the fourth survey of the Tromsø Study. Ultrasonographic imaging of the right carotid artery was used to assess prevalence of plaque and total plaque area (TPA), both at baseline, and after seven years of follow-up. This allowed us to study the direction of the association. Subjects were divided in RDW tertiles, and generalized linear models were used to analyze change in TPA across these categories. Novel plaque formation occurred in 40.2% of the subjects during follow-up, ranging from 33.2% of the subjects in RDW tertile 1 to 50.5% of the subjects in the highest tertile. Change in TPA was significantly higher across tertiles of RDW in multivariable analyses adjusted for BMI, total cholesterol, HDL cholesterol, systolic blood pressure, self-reported diabetes, smoking, platelet count, white blood cell count and hs-CRP levels. In analyses of RDW as a continuous variable, a 1% increase in RDW was associated with 0.6 mm^2 (0.1-1.2) increase in TPA in the multivariable model. Our findings suggest that RDW is associated with formation and progression of atherosclerotic plaques in the general population, independent of common atherosclerotic risk factors and hs-CRP. This might explain the link between RDW and arterial cardiovascular morbidity and mortality.

4.4 Paper IV

IMPACT OF CHRONIC INFLAMMATION, ASSESSED BY HS-CRP, ON THE ASSOCIATION BETWEEN RED CELL DISTRIBUTION WIDTH AND ARTERIAL CARDIOVASCULAR DISEASE: THE TROMSØ STUDY

We aimed to investigate whether the association between RDW and arterial CVD was confounded by chronic inflammation, or if RDW could be in the causal pathway between inflammation and arterial CVD. Study participants were recruited from the fourth survey of the Tromsø Study (Tromsø 4), a large population-based cohort. The study included 5756 individuals attending the second phase of Tromsø 4, which included measurements of high sensitivity C-reactive protein (hs-CRP). Baseline characteristics, including RDW and hs-CRP, were obtained in 1994/95, and participants were followed until December 31st 2012. Cox regression models were used to calculate HR with 95% CI for incident MI and ischemic stroke across quintiles of RDW and hs-CRP. Subjects with hs-CRP in the highest quintile had 36% higher risk of MI (HR: 1.36, 95% CI 1.08-1.72), and 56% higher risk of ischemic stroke (HR: 1.56, 95% CI 1.12-2.17) compared to subjects in the lowest quintile. Addition of RDW to the multivariable model attenuated the risk estimate for ischemic stroke slightly. Mediation analyses indicated that 6.8% (95% CI 3.6-30.9%) of the association between hs-CRP and ischemic stroke was mediated by RDW. Subjects in the highest RDW quintile had a 20% higher risk of MI (HR: 1.20, 95% CI 0.95-1.51) and 47% higher risk of ischemic stroke (HR: 1.47, 95% CI 1.05-2.04), compared to subjects in the lowest quintile. Addition of hs-CRP to the multivariable model slightly attenuated these estimates. In conclusion, our findings displayed a weak impact of hs-CRP on the association between RDW and arterial CVD, and that RDW had a modest direct effect on the risk of ischemic stroke, but not MI.

5. General discussion

5.1 Methodological considerations

5.1.1 Study design

The results from all papers in the present thesis are based on data from a population-based cohort. Through follow-up of the study population, the cohort study design is ideal for studying the natural history of disease. Participants free of a disease of interest are followed to estimate incidence rates, and to study associations between exposure and disease outcome.[202] Cohort studies are not suitable for investigations of rare conditions because it would require a very large study population, and a very long follow-up time to get a sufficient number of events. However, all the disease outcomes used in the papers of the present thesis, including MI, stroke and carotid atherosclerosis, are common in the general population. This makes the cohort design ideal to study our aims. The temporal sequence of the cohort study, with measurements of the exposure prior to outcome, is a clear advantage of this study design. It allows the investigators to conclude on whether the exposure or the outcome came first.[203] This is opposed to case-control or cross-sectional studies, which are prone to reverse causation, and lack the ability to assess cause-effect-relationships. RCTs and Mendelian randomization are considered the best ways to study causality. However, it is yet unknown how to intervene on an increased RDW, and no particular genetic variants associated with high RDW are known. Thus, for the time being, the cohort design is the best way to study the association between RDW and future development of disease. The cohort study also gives the opportunity to investigate several effects of a single exposure at the same time, in contrast to a case-control study where the research is limited to the selected cases. Depending on the participants, cohort studies might generate generalizable results. This entails that a

large number of subjects from a general population are included in the study. Some challenges to the cohort design merits consideration, including loss to follow-up and changes in disease risk and treatment during follow-up. Bias and confounding are also important issues, and will be discussed in more detail in the following paragraphs.

5.1.2 Selection bias

Systematic errors in recruitment and follow-up of the study population might cause an inaccurate representation of the target population, and selection bias occurs when these inaccuracies disturb true associations between exposure and outcome.[204]

A common error causing selection bias arises from the recruitment and attendance rate of health surveys. The fourth survey of the Tromsø study, used in all four papers of the present thesis, invited all inhabitants of the municipality of Tromsø aged 25 years or older. The participation rate was 77%, which is considered high.[197] A high attendance rate with subjects from a general population increases the generalizability of the results, and reduces the risk of selection bias. It has been argued that due to the temporal nature of the cohort study, the invited subjects do not know whether or not they will go on to suffer the outcome of interest, which limits the potential for bias due to low participation.[205] Nevertheless, the concept of self-selection might still limit the results, as the researcher do not control who the non-attendees are (23% in Tromsø 4). Previous studies show that participants of health surveys tend to have higher education and a healthier lifestyle compared to the non-responders.[206, 207] In paper III and IV, the study population was recruited from the second phase of Tromsø 4 with 76% of the eligible population attending. These participants were thereby exposed to two “rounds” of such self-selection bias.

Loss of study participants during follow-up may cause unwanted alterations to the study population. Such loss to follow-up rarely occurs at random, and is an important source of selection bias to keep in mind, as it may affect the exposed and unexposed participants differently and thereby impact the risk of the outcome of interest.[208] Migration is a common reason for loss to follow-up. As there is no reason to expect that people moving from Tromsø have a greater risk of arterial CVD than the people staying in Tromsø, simple censoring is an adequate way to handle these subjects. Once a participant move, the survival time is censored from that date. Such censoring is conducted in all four papers. Subjects dying during follow-up are censored in the same way. However, it is argued that death should be considered a competing event, especially in study populations of elderly subjects.[209] A competing event is an alternative outcome of equal or greater significance than the primary outcome that alter the probability of the outcome of interest.[210] RDW is associated with all-cause mortality in prospective studies of subjects recruited from the general population,[211, 212] and death could be a potential competing event in all four papers. In our material, there is a trend of increasing risk of death with higher RDW. However, this relationship is not strong enough to believe that it would substantially impact our results. Another potential competing event in paper I and IV is PCI conducted on patients with stable CAD. Such a procedure could reduce the risk of a future MI. However, a meta-analysis of RCTs on stent implantation versus medical therapy for stable CAD showed no benefit of PCI for prevention of death or nonfatal MI. [213] Thus, PCI does not appear to be an important competing event for MI in our material.

5.1.3 Information bias

Information bias occurs if definition, collection, analysis, or interpretation of study data is systematically inaccurate causing misclassification of either exposure or outcome.[204]

Misclassification bias is divided into two main types: *differential* and *non-differential*. If the misclassification of exposure variables varies within the study population, it is called differential.[214] Studies including baseline information obtained through self-administered questionnaires are vulnerable to misclassification. Inaccurate recollection of medical history, family history of disease and lifestyle factors may lead to misclassification of exposure variables. In case-control studies, with collection of baseline information after the cases and controls are chosen, there is a big potential for differential misclassification. This is due to the concept of *recall bias*, which implies that the cases and the controls might recall their past medical history and lifestyle risk factors in a different way. However, with a prospective cohort design, baseline information is obtained before the outcome occurs and recall bias is thereby eliminated. Potential misclassification in our studies will most likely be non-differential.

Several of the variables on life-style factors and medical history used in paper I-IV were obtained through self-administered questionnaires. Subjects reporting of arterial cardiovascular disease prior to study start were excluded from our analyses, while self-reported diabetes status was used in the multivariable adjustments. Studies show a high validity of self-reported history of MI and stroke, while a little worse for diabetes.[215, 216] A potential non-differential misclassification of diabetes status might be present, as other studies on diabetes prevalence report far higher numbers than the modest 1.7% reported by the Tromsø 4 participants.[146] Self-reported smoking status,

used for stratification and/or regression adjustments in all four studies, has also shown to give accurate estimates of true smoking prevalence.[217]

Measurement error is another potential source of non-differential misclassification. Measurement of RDW, the main exposure of interest in all four papers, may be exposed to technical errors. However, such potential errors would be random, not systematic, and are expected to only have a small, non-differential impact on the Tromsø 4 population. The analytic variation of RDW measurements was reported to less than 3% in Tromsø 4.

Non-differential misclassification might lead to a type of information bias called *regression dilution bias*. In the material used in the present thesis, all covariates are measured only at baseline. With only a single measurement, the regression model "assumes" that variables like smoking status, cholesterol level, and dietary habits, stay the same throughout the whole study period. If the exposure variable is misclassified at baseline, or if it changes during follow-up, it might lead to a dilution of the true effect of the exposure, reducing the strength of the observed associations.[124, 218] In 26 individuals with monthly blood samples, the intra-individual variation in RDW was reported to 3.4% over 1 year.[42] If accessible, repeated measurements of RDW could have limited a potential dilution of our risk estimates. Unfortunately, repeated measures of RDW was not available in our study population. Many of the cardiovascular risk factors are modifiable, and thereby prone to misclassification. In most cases, non-differential misclassification of covariates included in the multivariable regression models will lead to a diminished effect of the adjustment for the potential confounding.[219] In all four papers, RDW and all covariates of interest were collected only at baseline. How much the potential regression dilution will affect our results is uncertain. A recent study from the Tromsø 4 population showed good correspondence

between risk estimates for MI based on baseline and repeated measures of atherosclerotic risk factors.[220] Although repeated measurements of both exposure variables and potential confounders would be preferable, this study indicates that regression dilution is not a major source of bias in the four papers of this thesis.

5.1.4 Missing data

Despite cautious planning and execution of studies, there is no way to eliminate the possibility of missing data completely. Equipment failure (e.g. weight scale), error in laboratory handling, and inadequate response to self-administered questionnaires are just a few examples of how data might go missing.[221] Whether or not the missing data will introduce a significant bias largely depends on its magnitude, and if the data is missing randomly among the study participants. There are several ways to handle missing data to avoid biased associations. Many consider *imputation* to be the best way of doing this.[222] When performing imputation, a regression model estimates the underlying distribution of the variable with missing values, based on other known covariates in the study population. Subsequently, a value is randomly drawn from this distribution and imputed for the missing value. Single and multiple imputation refers to the use of a single or several different plausible imputed datasets. For instance if a subject lacks data on total cholesterol, a value can be estimated and imputed based on the cholesterol levels of study participants of the same age and sex, with similar triglyceride levels, blood pressure, BMI and HDL cholesterol. However, a study has shown that only 21% of the variation in RDW could be explained by commonly available factors such as age, sex, race, smoking, blood pressure, white blood cell count, BMI and hemoglobin.[223] Hence, estimation of RDW levels would probably be inaccurate, and imputation would not do the job. In all four studies of the present thesis,

we first excluded all subjects with missing data for any of the exposure or outcome variables. Secondly, we carried out *complete case analysis*, where the regression model automatically exclude subjects with a missing value for any of the covariates included in the multivariable model (i.e. the incomplete cases).[221] Therefore, the number of study participants in the various adjustment models of our regression analyses varied slightly depending on the covariates included (<2% missing). Complete case analysis is considered an acceptable approach leading to unbiased associations when the magnitude of the missing data is low, and when the data is considered to be missing completely at random. In our study, subjects with or without (2.3%) RDW values had equal proportions that developed an MI or stroke event during follow-up. Subjects with missing values of RDW also had missing data on MCV and Haemoglobin. The cause of these missing values is most likely insufficient quality of the blood samples/anticoagulant effect, an error believed to occur completely at random among the study participants.

5.1.5 Confounding and interaction

When assessing associations in cohort studies, confounding will always be a potential problem. Confounding is present when a non-causal association between a given exposure and an outcome is observed as a result of the influence of a third variable, which affects both the exposure and the outcome.[204] A general rule is that the confounding variable must be causally associated with the outcome. Furthermore, the confounder must be differently distributed among the groups compared in the study, and it cannot be an intermediate step in the causal pathway between exposure and outcome. Whether confounding classifies as a form of bias is discussed. An important thing to note when confounding is present, is that the observed association between

exposure and outcome might still be true, just not causal. Contrary, in the case of bias, there might be no reason to believe the association is true. Confounding can lead to both overestimation and underestimation of true associations. In cohort studies, differences between comparison groups may produce confounding, and the non-randomized nature of the study design makes it especially vulnerable to unmeasured and unrecognized confounders.[224]

Confounding should be controlled to ensure that the observed association is not due to an uneven distribution of other risk factors. The ideal way to do this is to have a randomized population, where the subjects have identical values for all independent variables other than the exposure, like in a RCT. However, most cohort studies have a non-randomized design, including the one used in the present thesis. To minimize confounding in our work, we used two different approaches: multivariable regression and stratification.[225] The regression analysis allows for inclusion of various independent variables in the multivariable model. By including the potential confounding variables in the analyses, the regression model calculates regression coefficients for all variables, adjusted for all other variables in the model.[226] Thereby it estimates the effect of the exposure on the outcome in light of the confounders. The selection of which covariates to include in the multivariable model is challenging. This selection should be based on available literature and knowledge about the mechanisms involved in the pathway between exposure and outcome. As these mechanisms are largely unknown with regard to RDW and arterial CVD, it is likely to think that some excess covariates are included in our analyses. Large prospective cohort studies have shown that RDW is associated with age, smoking, BMI, platelet count and cholesterol, all well-known risk factors for arterial CVD.[227, 228] Various inflammatory markers have also been shown to correlate moderately with RDW.[229, 230]

All these factors might potentially confound the association between RDW and arterial CVD. Our results remained significant after adjustments for age, smoking, BMI, cholesterol and platelet count. Papers III and IV also included adjustments for hs-CRP, which had a minimal impact on the association between RDW and arterial CVD. With adjustments, there is a possibility of overadjustment bias.[231] It is defined as control for an intermediate variable, or a descending proxy for an intermediate variable, on a causal path from exposure to outcome. When adjusting for a given variable, this will always include a partial adjustment for other variables related to it. In our analyses, there might be an issue with the adjustment for BMI, cholesterol, diabetes and hypertension. As previously discussed, the CVD risk associated with body weight is partly mediated through an increase in other atherosclerotic risk factors. Thus, the inclusion of BMI in our multivariable models could represent some overadjustment, but as BMI is weakly associated with RDW, it is not likely to have any substantial effect on our results.

When the effect of an exposure variable on a given outcome varies depending on a third variable, there is a statistical interaction present. A way of handling such interaction is by stratification, which is also commonly used to handle confounding. Stratification is the process of subdividing the study population into groups depending on status of different characteristics expected to influence the analyses. One problem with stratification is reduction of study power, as it might leave one of the subgroups with too few cases to evaluate the hypothesis sufficiently. Another problem is that stratification might leave other potential confounders unevenly distributed between the subgroups. However, stratification is usually combined with adjustments for other factors by regression as described above. In paper I, there was a statistical interaction between RDW and smoking status on the risk of MI. In addition to conventional

adjustments for smoking status, the study population was stratified according to smoking. The risk estimates for incident MI by RDW were higher among smokers than among nonsmokers, but the difference in HRs was not statistically significant.

Age is a common and important confounder in epidemiologic research, and is an issue in all four papers of this thesis. There are several ways to control for age in cohort studies. Traditionally, time-on-study has been used as the time-scale in Cox regression models. However, age can also be used as the time-scale, and this is thought to be a superior way of eliminating confounding by age, compared to stratification or age adjustments.[232] This is because the effect of age as a risk factor, in many diseases, is completely absent in the young while very strong in the old. The proportional hazard assumption states that the hazard a study participant faces is proportional to the baseline hazard function through time. For many diseases this assumption does not hold true for age. If the hazard of the outcome of interest is expected to change more as a function of age than as a function of time-on-study, it should be considered to use age as time-scale.[232] When using age as a time-scale, the events are compared with the participants who have not experienced an event or been censored by age *a*, instead of to those who are still in the study at time *t* counted from the date of inclusion. The proportional hazard assumption can be tested using Schoenfeld residuals.[233] Age as time-scale was used in papers I and IV.

Preventive measures to minimize confounding, including age as time-scale, multivariable regression and/or stratification, was conducted in all four papers, but residual confounding can never be completely ruled out.[234] There are various reasons for this, including poor measurement of confounding variables, unrecognized confounders, or large variations of the confounding variables within strata.[235]

5.1.6 Generalizability

The generalizability of a study refers to how applicable the results are to the rest of the local, national and global population.

Internal validity describes how well the study population reflects the population it is derived from, and the ability of the study to measure what it sets out to do.[236] Does the link between RDW and arterial CVD in the Tromsø 4 population adequately mirror the situation in the general population of the municipality of Tromsø? Selection bias, as previously discussed, might limit the internal validity. The whole population (≥25 years) was invited to Tromsø 4, and the survey had a high attendance rate, which strengthens the internal validity. The attendance rate was highest for subjects aged 40-80 years, and the generalizability of the findings might thereby be weaker for inhabitants <40 and >80 years.[197] Paper I and IV in the thesis are based on the second phase of Tromsø 4. To this second phase, the participants were selected based on age with a majority aged 50-74 years, and only small samples (5-8%) from other 5-year age intervals. This weakens the generalizability of the results for the underrepresented subgroups.

External validity says something about how well the results can be extrapolated to other populations.[236] The vast majority of the Tromsø 4 participants, and the general Tromsø population, are white Caucasians. As previously described, there are large differences in the incidence of MI and stroke depending on ethnicity.[60, 63-65] This weakens the generalizability of our results to non-Caucasian populations. The measurement and calculation of RDW is not standardized, and this weakens the external validity of the results by a great deal. For instance, in the Malmö Cancer and Diet cohort, which is comparable to Tromsø 4 in size and findings, RDW was calculated and expressed in a different way.[227] In the Malmö cohort, RDW was calculated from

the width of the erythrocyte volume distribution curve at a relative height of 20% above the baseline, presented as femtoliters. This expresses the standard deviation, and does not take into account the MCV.[36] A study comparing four hematological analyzers found a lack of harmonization in RDW values.[237] When setting a threshold value to RDW>14.6%, the number of subjects with values exceeding this was significantly different among the four hemocytometers. Still, the imprecision within the different hemocytometers was only between 0.3-1.2%. This indicates that the internal validity will not be affected as long as the same analyzer is used for the whole study population. Our results might be extrapolated to similar populations in the sense that high RDW increases the risk of arterial CVD. However, before the measurement of RDW is standardized, it is not expedient to create cut-off values for RDW associated with high risk.

5.1.7 Outcome registration and validation

All first-time MI and stroke events occurring during follow-up were retrospectively identified by linkage to various registries, as previously described. UNN is the only hospital serving the Tromsø region, and the chance of the MI and stroke registry being complete is thereby high. Still, some events might have been diagnosed and treated in other hospitals than UNN. To reduce the potential for misclassification of events, trained personnel recorded and validated all events following strict criteria. However, outcome misclassification can never be completely ruled out. Complete registration of events is dependent on accurate information and diagnosis coding in the medical records.

The registration of atherosclerosis was dependent on the different sonographers using standardized examination techniques and measurement procedures. Compared

to the MI and stroke events, the atherosclerosis outcome is more prone to misclassification due to individual differences among the sonographers. The between- and within- sonographer reproducibility of ultrasound assessment of plaque occurrence and thickness has been investigated in the Tromsø study.[198] The study found good agreement both between- and within- sonographer on plaque occurrence, but only moderate reproducibility of plaque thickness. In the present thesis, we investigated change in TPA, and did not use data on IMT. The TPA was calculated from still images recorded of each plaque. Hence, our analyses rely on the plaque data with the best between-sonographer reproducibility. Another potential shortcoming is that only the right carotid artery was examined. Inclusion of the left carotid artery, as well as the femoral arteries, could have given a better impression of the individual plaque burden. However, a study comparing the two carotids intra-individually suggested that the disease was bilaterally symmetrical.[238]

5.2 Discussion of main results

5.2.1 The association between RDW and arterial CVD (paper I, II and III)

In paper I and II, we reported that RDW was associated with an increased risk of incident MI and stroke in a prospective cohort with subjects recruited from the general population. The results were independent of common atherosclerotic risk factors and anemia. Prior to the present thesis, the evidence regarding RDW and arterial CVD was of a more suggestive nature. Design and study population limited these studies, making them unable to say anything about the direction of the association. A few studies with varying agreement to our findings have emerged over the last few years. Li et al. described a relationship between RDW and higher Framingham risk score for CAD events in 392 patients undergoing coronary

angiography.[239] In 2014, Borné et al. published results from a prospective study on RDW and coronary events,[228] including 26820 participants from the Malmö Diet and Cancer cohort. During a mean follow-up of 14 years, 1995 subjects suffered a first-ever coronary event, defined as an acute MI or death due to ischemic heart disease. Subjects in the highest RDW quartile had a 20% higher risk of a coronary event compared to subjects in the bottom quartile (HR: 1.20, 95% CI 1.06-1.37). After subdivision of the cases, they found that the fatal coronary events were driving this association, with a 1.8-fold higher risk (HR: 1.82, 95% CI 1.35-2.44) in subjects with top quartile values of RDW. There was no relationship between RDW and non-fatal coronary events.[228] A potential difference between fatal and non-fatal MIs was not investigated in our study. Findings from the Malmö cohort with regard to RDW and stroke were more in line with our results. Söderholm et al. found that subjects in the highest RDW quartile had a 31% higher risk of stroke (HR: 1.31, 95% CI 1.11-1.54) when compared to the lowest quartile.[227] The cerebral infarctions were driving this association, with a HR of 1.32 (95% CI 1.10-1.58) when comparing top with bottom RDW quartile.[227] This is also in line with our findings, as we found no association between RDW and hemorrhagic stroke events. Saliba et al. described a very similar risk estimate in patients with atrial fibrillation followed for 1 year. Subjects in the highest RDW quartile had a HR for stroke of 1.33 (95% CI 1.15-1.53) when compared to the lowest quartile.[240]

Atherosclerosis is a shared risk factor that might explain the observed relationships between RDW, MI and stroke, as it plays an important role in the development of both.[96, 97, 110] In paper III, we reported that RDW was significantly associated with novel plaque formation and a larger mean change in TPA over a 7-year period. To the best of our knowledge, no other study has evaluated RDW and

progression of atherosclerosis in a prospective cohort with subjects recruited from the general population. However, several cross-sectional studies of selected populations support our findings, showing increased prevalence and severity of atherosclerosis with increasing RDW.[188-190] In 156 hypertensive patients aged 60-85 years, the carotid plaque prevalence and IMT to inner diameter ratio was significantly higher among subjects with RDW values in the highest quartile.[188] A cross-sectional study of 6950 subjects aged 40 years or older found a graded increase in prevalent PAD, assessed by ankle-brachial index, with increasing RDW quartiles.[190] In 193 non-anemic patients undergoing coronary angiography for stable angina pectoris, the subjects with CAD had significantly higher RDW values, and RDW correlated with the severity of the coronary disease assessed by SYNTAX.[189] In the Malmö Diet and Cancer study, carotid artery ultrasound was conducted on a subcohort (n=5309) randomly selected from the population-based cohort (n=26879). They found a significantly higher common carotid artery IMT in the highest RDW quartile, compared to the lowest.[227] This study is similar to ours in design and size, but the subcohort in the Swedish study did not undergo a follow-up ultrasound of the carotid artery, and they were unable to investigate progression or de novo formation of atherosclerotic plaques after RDW measurement.

The underlying mechanism for the association between RDW and arterial CVD remains unknown. Several theories exist, but few of them have been thoroughly investigated. Viewed roughly, the relationship might be explained by either an *indirect* or a *direct* role of RDW in disease development. An indirect relationship could for instance be due to a confounding factor, affecting both exposure and outcome (Figure 4), where RDW has no causal role in disease development.

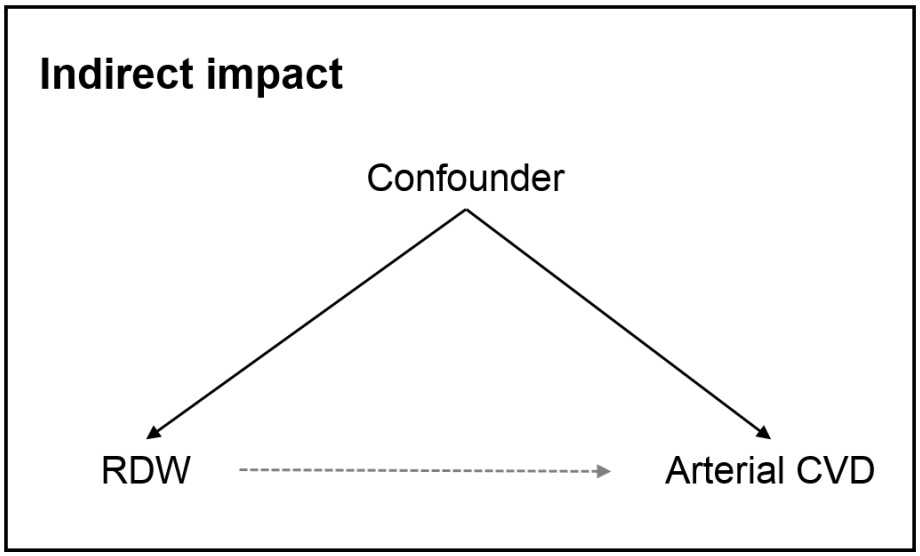

Figure 4. RDW could have an indirect impact on disease development by being a marker of something else with unknown confounding factors causing apparent associations.

On the other hand, RDW could be a proxy marker for a direct and causal effect of the erythrocyte size distribution in the pathogenesis of arterial CVD (Figure 5). This could be by RDW acting as a mediator of the causal pathway between known causal risk factors and arterial CVD (Figure 5A). Another possible way of RDW affecting the pathogenesis directly is through an effect of RDW on risk factors common to MI and

ischemic stroke (Figure 5B). One example of such a common risk factor is atherosclerosis, as previously discussed.

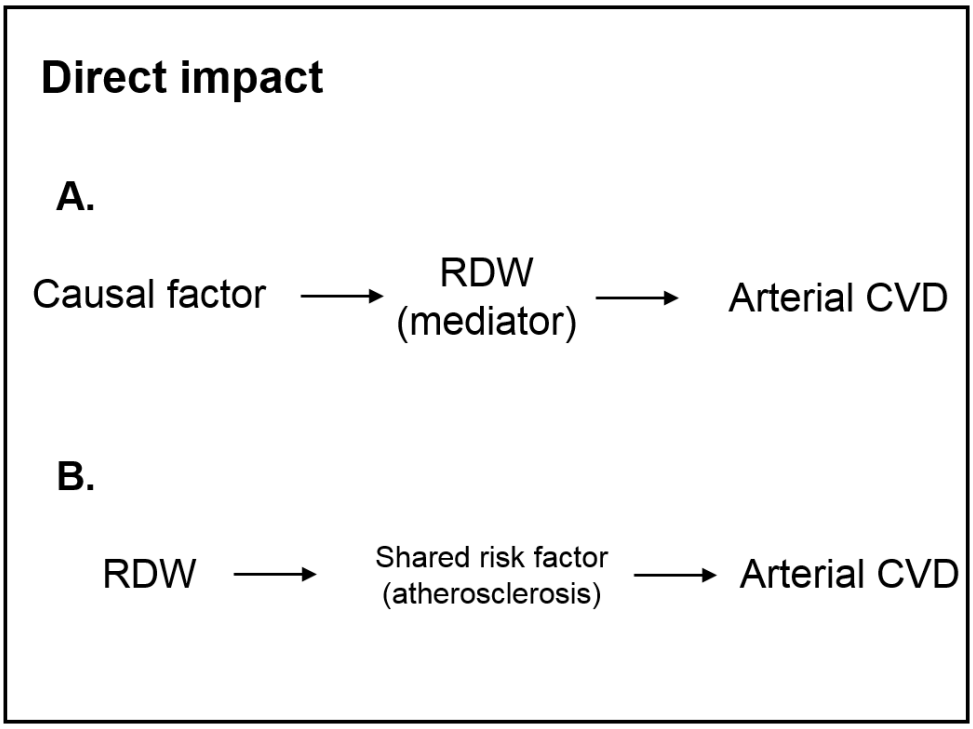

Figure 5. Erythrocyte size variation, with RDW as a proxy marker, might act directly on the causal pathway to disease. RDW could be a mediator of some other causal factor (5A), or it could be causing changes in shared risk factors for MI and ischemic stroke, like atherosclerosis (5B).

In 1965, Sir Bradford Hill purposed nine criteria to use in evaluation of whether an observed association could be due to a causal relationship or not.[241] Based on the results from cohort studies, we cannot conclude on whether RDW play a causal role in disease development. However, the findings can be discussed and substantiated in the light of Hills nine criteria:

Temporality states that the exposure must precede the outcome for the relationship to be causal. This is considered the only absolute criteria for causation. The cohort study design facilitates temporality. Exposure, in our case RDW, is measured at baseline in initially disease-free subjects, which are followed until an event. This criterion holds true for all four papers in the present thesis. In papers I, II and IV we studied incident events after exclusion of all subjects who had experienced

the disease of interest prior to baseline measurements. In paper III, we studied growth of atherosclerotic plaques as well as de novo plaque formation in subjects both with and without carotid plaques at baseline.

The *strength of the association* is considered important in assessment of causality. The stronger an association is, the less likely it is to be caused by residual confounding, bias or by random.[236] It is suggested by Szklo and Nieto that a relative risk below 2.0 should be considered a weak association in evaluation of causality.[204] In paper I and II we found that RDW values in the highest quintile was associated with a 1.3-1.4-fold higher risk of MI and stroke, when compared to the bottom quintile. Although significant, this is viewed as rather modest associations. However, cholesterol levels, a recognized causal factor in arterial CVD development, also show relatively low risk estimates. A study compared the relationship between total cholesterol and long-term mortality from CHD in cohorts from seven different countries.[242] The relative risks for the highest compared to the lowest cholesterol quartile ranged from 1.1-2.3. This study undermines the importance of the strength criterion.

The c*onsistency* criterion says that the association must be confirmed in other studies with different study populations. If a relationship truly is causal, it should be observed in a consistent fashion in all studies investigating the association. This criterion is especially exposed to publication bias, as studies showing negative associations have a tendency to stay unpublished.[243] The available data on RDW and MI is inconsistent. Two prospective cohort studies, one from Taiwain, and the other one from Malmö, Sweden, showed results that are in contrast with our findings. Chen et al. found no association between RDW and future CVD morbidity or mortality,[43] while Borné et al. found no association between RDW and non-fatal coronary events.[228]

Published data on RDW and stroke is more consistent throughout, with the study from Taiwan being one exception.[43] Studies consistently show an association between RDW and atherosclerosis, but except for paper III in the present study, they all have a cross-sectional design and are therefore less useful in discussion of causation.

The *biological gradient* criterion says that if causal, there should be a dose-response relationship between exposure and outcome, and not just a threshold effect. In all four papers, we found a significant dose-response relationship between RDW and arterial CVD outcomes.

The *specificity* criterion states that the exposure should be specifically associated with the outcome of interest for it to be a causal relationship. This is not true with regard to the relationship between RDW and arterial CVD, as RDW is associated with a wide specter of disease entities, e.g. cancer and venous thrombosis.[244, 245] However, this is considered a weak criterion, as very few causal factors lead to only one specific outcome.[236]

The *plausibility* and the *coherence* criteria says that an association should be biologically plausible, and not in conflict with current knowledge, for it to be causal. Possible ways of a direct impact of erythrocyte size distribution on the development of arterial CVD will be discussed later in the thesis.

The *experiment* criterion argue that an association, if causal, should be proven by an experimental intervention. A randomized controlled trial (RCT) would be the way to do this. However, as it is still unknown how to intervene on RDW (in non-anemic subjects), it has never been tested in an RCT. A study comparing different study designs found that the results from well-designed observational studies and RCTs

were remarkably similar.[246] This supports the quality of the evidence from large cohort studies.

The *analogy* criterion refers to the assessment of an association based on what has previously been proven in similar situations. To the best of our knowledge, no causal relationship between RDW and disease outcome of any kind has been proven thus far.

Except for temporality, failure to satisfy any of Hills criteria does not exclude the possibility of a causal association. With regard to evaluation of RDW as a causal factor for arterial CVD, several of the criteria are fulfilled, but there are also large prospective studies with conflicting results, which undermines a causal relationship. In the following two sections, possible underlying mechanisms for both an indirect and a direct/causal relationship between RDW and arterial CVD will be discussed.

5.2.2 Indirect mechanisms explaining the relationship between RDW and arterial CVD (Paper I-IV)

In light of Hills criteria, it seems plausible that an explanation for the association between RDW and arterial CVD could lie in an indirect, non-causal relationship with other common/confounding factors involved. Several factors could potentially explain an indirect relationship between RDW and arterial CVD.

Inflammation affects red blood cell life span and modulates the effect of erythropoietin on the erythropoiesis, and can thereby affect RDW.[247] As previously described, inflammation play an important role in the pathogenesis of arterial CVD, and CRP is an independent risk factor for MI and stroke.[3, 181] Several studies have reported relationships between RDW and various markers of inflammation. In a large cohort of 3845 unselected outpatients, subjects in the highest RDW quartile had 3-fold higher erythrocyte sedimentation rate (ESR) and hs-CRP when compared to subjects in the lowest quartile.[230] RDW correlated significantly with ESR ($r=0.457$, $p<0.001$) and CRP ($r=0.350$, $p<0.001$) in patients with Alzheimer.[248] A correlation between RDW and CRP has also been reported in other selected populations, including patients with non-dipper hypertension ($r=0.403$, $p<0.001$),[249] patients with coronary artery disease ($r=0.181$, $p<0.001$),[250] and in overweight adolescents ($r=0.241$, $p=0.034$).[251] In paper IV, simple correlations revealed a significant correlation coefficient ($r=0.13$, $p<0.0001$) between RDW and hs-CRP, but this would traditionally be considered as weak.[252] We specifically tested the role of chronic inflammation on the relationship between RDW and arterial CVD by including hs-CRP to the multivariable regression analyses in paper III and IV, but this had a very modest effect on the risk estimates. A few previous studies support these findings. The association between RDW and fatal coronary events reported in the Malmö Diet and Cancer study was left unchanged after addition

of leukocyte count to the analyses,[228] while CRP had no effect on the association between RDW and heart failure.[253] A 1% increment in RDW yielded an equal risk of all-cause mortality independent of hs-CRP levels.[254] Neither the correlations reported, nor the regression analyses accounting for an inflammatory state, support the theory of inflammation being an important confounder of the association between RDW and arterial CVD. The measurements of hs-CRP in our study, and the inflammatory markers used in other studies, might not sufficiently reflect a chronic inflammatory state. In paper IV, we partly addressed this issue by excluding all subjects with hs-CRP >10 mg/l, as we did not want to study subjects with an ongoing acute inflammatory response.[255]

Oxidative stress play a central role in in the pathogenesis of atherosclerosis.[256] Increased oxidative stress is stimulated by common atherosclerotic risk factors, including hypertension, hypercholesterolemia, diabetes and smoking.[80] Moreover, oxidative stress contributes significantly to the regulation of hematopoietic cell homeostasis and could thereby have an influence on RDW.[257] In a study following 786 moderately to severely disabled women aged ≥65 years, the antioxidant Selenium was associated with RDW both at baseline and at follow-up measurements after 12 and 24 months.[258] Oxidative stress could be an important confounding factor of the association between RDW and arterial CVD. Unfortunately, the data material we used in papers I-IV did not include measurements of reactive oxygen species, and we were thereby unable to test this hypothesis. Several of the other studies on RDW and arterial CVD also mention reactive oxygen species as a potential factor involved in the relationship. However, neither of these studies have been able to evaluate this theory.

Anemia is clearly linked to alterations in RDW. Additionally, in a prospective cohort of 14410 previously healthy subjects, anemia was independently associated

with an increased risk of CVD (HR: 1.41, 95% CI 1.01-1.95).[259] Anemia could act as a confounding factor of the relationship between RDW and arterial CVD. We tested this hypothesis in papers I, II and III by removing all subjects with anemia according to the World Health Organization's definition (hemoglobin<13 g/dl for men and <12 g/dl for women).[260] After exclusion of subjects with anemia in paper I (n=1297), paper II (n=1102) and paper III (n=100), the risk estimates were left essentially unchanged. Our finding that RDW is associated with atherosclerosis, MI and stroke independent of anemia is supported by several other studies. In the Malmö cohort, describing associations between RDW and stroke,[227] and RDW and fatal coronary events,[228] exclusion of anemic subjects had no significant effect on the risk estimates. Arbel et al. found that the association between RDW and risk of major cardiovascular events existed in both anemic and non-anemic participants, but that it was significantly stronger in the non-anemic.[31] The association between RDW and Framingham risk score presented by Zalawadiya et al. was also independent of anemia.[30] Thus, the relationship between RDW and arterial CVD seems to be independent of anemia.

Atrial fibrillation is an important risk factor for ischemic stroke. In the Malmö Diet and Cancer study, including 27124 subjects, RDW values in the highest quartile was associated with a 33% higher risk of AF (HR: 1.33, 95% CI 1.16-1.53) when compared to the lowest quartile.[261] This association might partly explain the relationship between RDW and ischemic stroke. However, in the study by Söderholm et al. from the same cohort, the association between RDW and stroke remained similar after exclusion and censoring of subjects with AF, prior to and during follow-up.[227] We did not investigate the potential effect of AF on our results.

To date, no clear explanation for an indirect relationship between RDW and arterial CVD has been identified. There might be different reasons for this. Firstly,

residual confounding by recognized factors may still be present. We hypothesized that chronic inflammation could confound the association. However, hs-CRP might not reflect a chronic inflammatory state sufficiently. We also recognize oxidative stress as a potential confounder, but the nature of the cohort design leaves us unable to take into account unmeasured variables. Secondly, residual confounding can arise from factors that are still not recognized, which quite obviously make it hard to control for these factors. Thirdly, if the relationship between RDW and arterial CVD is direct and causal, the explanation might not lie in a third confounding factor at all. Lastly, with all the common factors affecting both the erythropoiesis and atherosclerosis, it is very likely that the observed relationship between RDW and arterial CVD is influenced by a complex interplay between several factors, making it impossible to point out a single explanation.

5.2.3 Direct mechanisms explaining the relationship between RDW and arterial CVD (Paper IV)

The red blood cells are directly involved in atherosclerotic plaque progression, destabilization, and thrombus formation. Several lines of evidence support the theory that high intra-individual variation in the erythrocyte size could have a direct influence on these disease mechanisms, and thereby alter the risk of arterial CVD by directly affecting the causal pathway.

The erythrocyte membrane contains large amounts of cholesterol, 1.5-2 times more than any other cell membrane in the body.[262] It is shown that cholesterol from erythrocyte membranes contribute to growth of the necrotic core, and destabilization of the atherosclerotic plaque predisposing it to rupture.[263] This is supported by a study by Tziakas et al., where the total cholesterol content of the erythrocyte membrane was increased in patients with acute coronary syndrome, compared to patients with stable angina.[264] The total cholesterol content of the erythrocyte membrane is also associated with the severity of coronary artery disease.[265] An important source of this erythrocyte-containing cholesterol is intraplaque hemorrhage, an important factor in atherosclerotic plaque progression.[266, 267] Angiogenesis is associated closely with plaque progression, and intimal neovascularization is thought to be an important source of intraplaque hemorrhage.[268] In multivariable analyses, RDW levels are shown to be independently associated with increased cholesterol content of the erythrocyte membrane.[269] Subjects with high RDW suffering from intraplaque hemorrhage will therefore be prone to both an increased plaque growth and plaque destabilization. The results presented in paper III show an association between RDW and future growth of atherosclerotic plaque. A direct relationship between RDW and plaque growth is in line with the

findings in all four papers of the present study, fitting the model presented in Figure 5B.

Another potential way RDW could influence arterial CVD directly is through hypercoagulability and stasis. The results in paper IV indicate that RDW play a role as a mediator in the causal pathway between inflammation and ischemic stroke (Figure 5A). No other studies to date have conducted such mediation analyses. One potential explanation for a relationship where RDW acts as a proxy for erythrocyte features involved in ischemic stroke development, lies in hypercoagulability and stasis. The ability of the erythrocytes to deform is important to facilitate blood flow in the microcirculation, as it reduces red cell aggregation and flow resistance.[270] The flow properties of the erythrocytes affect oxygen supply to the tissue. In a study by Patel et al., RDW was associated with decreased red cell deformability.[271] Hence, an increased RDW might lead to increased red cell aggregation and hypoxia. The notion that RDW promotes aggregation fits well with a role of RDW in mediating the relationship between inflammation and ischemic stroke. Around 40% of the ischemic strokes are due to cardioembolic events, with altered flow properties and stasis being crucial in the pathogenesis. Growing evidence support a more pronounced role of hypercoagulability and stasis in the pathogenesis of ischemic stroke than in the pathogenesis of MI, which is in line with our findings in paper IV. Mechanisms related to hypercoagulability, such as prothrombotic genotypes, are reported having a greater effect on ischemic stroke than MI.[272] In addition, several studies have described an association between RDW and venous thromboembolism, which supports the theory that RDW might be directly involved in stasis and altered coagulation.[244, 273, 274]

Available evidence support different pathways in which the size variation of the erythrocytes play a causal role in arterial CVD development. However, some evidence

also contradict a direct and causal involvement of RDW in arterial CVD. Firstly, RDW is associated with a vast variety of disease entities, including several conditions with no relation to coagulation, like cancer stage,[275] inflammatory bowel disease activity,[276] non-alcoholic fatty liver disease,[277] and prevalence of dementia.[278] Secondly, in a study of 6689 patients undergoing PCI, major bleeding complications and need for transfusions were significantly higher in patients with higher baseline RDW.[279]

Whether or not high RDW have a direct or indirect role in the development of arterial CVD is still unknown. Available evidence, including what is added by the findings in the present thesis, point in different directions. It is likely that the explanation lies in an interplay between several factors.

6. Conclusions

- RDW was associated with incident MI in a prospective cohort study with participants recruited from the general population. The risk estimates were independent of traditional atherosclerotic risk factors and anemia.
- RDW was associated with a higher risk of incident stroke, independent of anemia and traditional atherosclerotic risk factors. The association between RDW and stroke was driven by the ischemic stroke events, with no observed relationship between RDW and hemorrhagic stroke.
- RDW was independently associated with prevalence and growth of carotid atherosclerotic plaques in subjects recruited from the general population.
- The association between RDW and carotid atherosclerosis, MI and ischemic stroke was not explained by a chronic inflammatory state, assessed by baseline hs-CRP values. RDW played a modest role mediating the relationship between hs-CRP and ischemic stroke.

7. Implications of results and future perspectives

During the last decades, there has been a substantial decrease in the incidence of arterial cardiovascular morbidity and mortality. Mortality rates of both stroke and MI are dropping due to improved extra- and intra-hospital treatment,[9, 10] while better primary prevention, including treatment for diabetes, hypertension and dyslipidemia, has reduced the incidence of MI events.[12] Still, MI and stroke remain among the most common causes of death worldwide.[71]

An important step in improved disease prevention is identification of novel risk factors. RDW is inexpensive and easily accessible, and could be a good candidate for such a marker. Although it is still unknown whether RDW represent a direct or indirect role in disease development, it might not be necessary to understand this to utilize RDW in risk stratification. Our studies from the Tromsø population, as well as other large prospective studies, have shown that RDW is associated with incident MI and stroke independent of common cardiovascular risk factors. Thus, RDW could potentially add to the value of factors such as BMI, hypertension, cholesterol and smoking in assessment of patients at high risk of cardiovascular events.

The underlying mechanism for the association between RDW and CVD should be further explored. We are currently investigating the impact of iron metabolism and iron deficiency on the association between RDW and venous thromboembolism in a nested case-control study derived from the Tromsø Study. The Tromsø Study also has information on AF, and we could further explore the association between RDW and AF, and how AF influences the association between RDW and ischemic stroke. Furthermore, the potential role of oxidative stress in the relationship between RDW and arterial CVD is still an unwritten chapter. Future studies are warranted to investigate this.

8. References

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Paper I

Paper II

Paper III

Paper IV

Appendix

Innbydelse til

HELSEUNDERSØKELSEN

Fødselsdato	Personnr.	Kommune	Kretsnr.

Velkommen til helseundersøkelsen i Tromsø!

Helseundersøkelsen kommer nå til Tromsø. Tid og sted for frammøte finner du nedenfor. Du finner også en orientering om undersøkelsen i den vedlagte brosjyren.

Vi ber deg fylle ut spørreskjemaet på baksiden og ta det med til undersøkelsen.

Undersøkelsen blir mest verdifull om frammøtet blir så fullstendig som mulig. Vi håper derfor at du har mulighet til å komme. Møt selv om du kjenner deg frisk, om du er under legebehandling, eller om du har fått målt kolesterol og blodtrykk i den senere tid.

Vennlig hilsen
Kommunehelsetjenesten
Fagområdet medisin, Universitetet i Tromsø
Statens helseundersøkelser

EGEN HELSE

Hvordan er helsen din nå? *Sett bare ett kryss.*

Dårlig	12	1
Ikke helt god		2
God		3
Svært god		4

Har du, eller har du hatt:

	JA	NEI	Alder første gang
Hjerteinfarkt 13			år
Angina pectoris (hjertekrampe) 16			år
Hjerneslag/hjerneblødning 19			år
Astma 22			år
Diabetes (sukkersyke) 25			år

Bruker du medisin mot høyt blodtrykk?

Nå	28	1
Før, men ikke nå		2
Aldri brukt		3

Har du i løpet av det siste året vært plaget med smerter og/eller stivhet i muskler og ledd som har vart i minst 3 måneder sammenhengende? 29 — JA / NEI

Har du de siste to ukene følt deg:

	Nei	Litt	En god del	Svært mye
Nervøs og urolig? 30				
Plaget av angst? 31				
Trygg og rolig? 32				
Irritabel? 33				
Glad og optimistisk? 34				
Nedfor/deprimert? 35				
Ensom? 36				
	1	2	3	4

RØYKING

Røykte noen av de voksne hjemme da du vokste opp? 37 — JA / NEI

Bor du, eller har du bodd, sammen med noen dagligrøykere etter at du fylte 20 år? 38 — JA / NEI

Hvis "JA", hvor mange år tilsammen? 39 — Antall år

Hvor lenge er du vanligvis daglig tilstede i røykfylt rom? 41 — Antall timer

Sett 0 hvis du ikke oppholder deg i røykfylt rom.

Røyker du selv:

	JA	NEI
Sigaretter daglig? 43		
Sigarer/sigarillos daglig? 44		
Pipe daglig? 45		

Hvis du har røykt daglig tidligere, hvor lenge er det siden du sluttet? 46 — Antall år

Hvis du røyker daglig nå eller har røykt tidligere:

Hvor mange sigaretter røyker eller røykte du vanligvis daglig? 48 — Antall sigaretter

Hvor gammel var du da du begynte å røyke daglig? 52 — Alder år

Hvor mange år tilsammen har du røykt daglig? 54 — Antall år

MOSJON

Hvordan har din fysiske aktivitet i fritiden vært det siste året? *Tenk deg et ukentlig gjennomsnitt for året.*
Arbeidsvei regnes som fritid.

	Timer pr. uke: Ingen	Under 1	1-2	3 og mer
Lett aktivitet *(ikke svett/andpusten)* 56				
Hard fysisk aktivitet *(svett/andpusten)* 57				
	1	2	3	4

KAFFE

Hvor mange kopper kaffe drikker du daglig?
Sett 0 hvis du ikke drikker kaffe daglig.

Kokekaffe	58	Antall kopper
Annen kaffe	60	Antall kopper

ALKOHOL

Er du total avholdsmann/-kvinne? 62 — JA / NEI

Hvor mange ganger i måneden drikker du vanligvis alkohol? *Regn ikke med lettøl.*
Sett 0 hvis mindre enn 1 gang i mnd. 63 — Antall ganger

Hvor mange glass øl, vin eller brennevin drikker du vanligvis i løpet av to uker? 65
Regn ikke med lettøl.
Sett 0 hvis du ikke drikker alkohol.

Øl	Vin	Brennevin
glass	glass	glass

FETT

Hva slags margarin eller smør bruker du vanligvis på brødet? *Sett ett kryss.*

Bruker ikke smør/margarin	71	1
Meierismør		2
Hard margarin		3
Bløt (soft) margarin		4
Smør/margarin blanding		5
Lettmargarin		6

UTDANNING/ARBEID

Hvilken utdanning er den høyeste du har fullført?

Grunnskole, 7-10 år, framhaldsskole, folkehøgskole	72	1
Realskole, middelskole, yrkesskole, 1-2-årig videregående skole		2
Artium, øk.gymnas, allmennfaglig retning i videregående skole		3
Høgskole/universitet, mindre enn 4 år		4
Høgskole/universitet, 4 år eller mer		5

Hva slags arbeidssituasjon har du nå?

Lønnet arbeid	73	
Heltids husarbeid	74	
Utdanning, militærtjeneste	75	
Arbeidsledig, permittert	76	

Hvor mange timer lønnet arbeid har du i uka? 77 — Antall timer

Mottar du nå noen av følgende ytelser?

Syketrygd (sykmeldt)	79	
Attføring	80	
Uførepensjon	81	
Alderspensjon	82	
Sosialstøtte	83	
Arbeidsløshetstrygd	84	

SYKDOM I FAMILIEN

Har en eller flere av foreldre eller søsken hatt hjerteinfarkt (sår på hjertet) eller angina pectoris (hjertekrampe)? 85 — JA / NEI / VET IKKE

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