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UiT / THE ARCTIC UNIVERSITY OF NORWAY

RED CELL DISTRIBUTION WIDTH (RDW) AND RISK OF ARTERIAL CARDIOVASCULAR DISEASE

- A Literature Study

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Summary

Background: Myocardial infarction is the leading cause of death worldwide. Stroke is the third most common cause of death in the western world, and the primary cause of permanent disability. Both diseases are, in the majority of the cases, a result of atherosclerotic plaque formation and rupture. Many common risk factors and preventive options have already been discovered for these diseases, but the incidence remains high.

Red cell distribution width (RDW) is a measure of the variability in size of the circulating erythrocytes. It is calculated by most common blood cell counters. Over the last few years it has been associated with a specter of disease entities including arterial cardiovascular disease. The underlying reason for the observed associations remain unknown.

Aim: The aim of this literature study is to give an overview of the epidemiology and pathophysiology of atherosclerosis, ischemic heart disease and stroke, and present the available literature on the relationship between RDW these diseases. Lastly, potential underlying mechanism for the observed associations will be discussed.

Method: I've approached the problem by use of scientific literature both published as books and relevant research articles. I collected the research articles from the MEDLINE database by using the medical search engines PubMed and Google Scholar.

Results: Many low-scale retrospective and some larger prospective studies have described relationships between RDW and myocardial infarction, stroke and atherosclerosis. There are also a few studies reporting the contrary. RDW has also been described as a prognostic marker for mortality after myocardial infarction.

Conclusion: RDW is related to atherosclerosis, myocardial infarction and stroke, and can potentially be used as a novel biomarker for risk stratification and prevention of disease. The underlying mechanism for the associations remains unknown, but in most of the literature RDW is proposed as a marker of an inflammatory state in the body.

Timeline of the project period

August 2015 (two weeks full-time)	Worked on the framework of the project and made an outline of the thesis regarding what to include and what to leave out. Got feedback from my supervisors on the outline and started collecting relevant literature from the MEDLINE database by using pubmed and google scholar.
September 2015 to April 2016	Reviewed the retrieved literature, and collected some more on topics where it was needed using the same method as described above. I started writing on a first draft on the association between red cell distribution width and risk of arterial cardiovascular disease based on the retrieved literature. Throughout this period I communicated with my supervisors regarding the content and direction of the thesis.
April through May 2016	I completed the first draft. I got feedback from my supervisors on the content of the thesis. They gave me advice on improvements regarding the content. I made changes and completed a second draft which I also got feedback on. The final draft was finished in May 2016. The project was more or less carried out according to the plan in the project description.

Introduction

Cardiovascular disease (CVD) is a collective term including all heart and blood vessel diseases. Diseases related to the process of atherosclerosis, like heart attack, stroke and peripheral arterial disease, are all included in the term, as well as disease entities like arrhythmias, heart valve problems and venous thrombosis. In this literature study, my focus is going to be on arterial cardiovascular diseases including ischemic heart disease, stroke and atherosclerosis.

Atherosclerosis, the process of development and rupture of atherosclerotic plaques, is the leading cause of myocardial infarction, ischemic stroke and peripheral artery disease (1-3). Ischemic heart disease due to atherosclerosis is the leading cause of death both worldwide and in Europe. It is estimated that CVD is responsible for over four million deaths yearly in Europe, which makes it attributable for about 51% of deaths in women, and 42% of deaths in men (4, 5). Many of the deaths due to CVD happens prematurely (<75 years), and the disease(s) accounts for 37% of all premature deaths in Europe. Over the last few decades the evolution of treatment and prevention of acute myocardial infarctions have markedly decreased disease mortality rates. Incidences, on the other hand, are more stable and even increasing among women in some populations (6). However, recent results from the same Norwegian population show that the incidence rates are decreasing as well (7). Cerebrovascular events, more commonly known as strokes, are the number one cause of permanent disability, and the third most common cause of death in the western world (8-10) Atherosclerosis plays a significant role in cerebrovascular events, especially the ischemic strokes (11). In addition to the arterial cardiovascular diseases, the CVD term includes arrhythmias, heart valve diseases, congenital heart disease, deep vein thrombosis, pulmonary embolism and rheumatic heart disease. These disease entities will not be discussed in this thesis.

Cardiovascular disease is without doubt an important public health problem, with ischemic heart disease and stroke on top of WHOs cause of death statistics (12). Prevention and early intervention is crucial to decrease these numbers. Except for genetic predisposition, gender and age, many of the commonly known risk factors for cardiovascular diseases are modifiable. Such risk factors include smoking, physical activity, alcohol consumption, diet, overweight, hyperglycemia, hyperlipidemia and hypertension (13-24). These can be controlled and treated with medication and/or by alteration of life-style. Identification of new risk factors is also an important step in the prevention of CVD. Biomarkers able to predict

cardiovascular outcomes can aid identification of subjects in need for early intervention, and thereby prevent fatal events. One such biomarker is Red blood cell Distribution Width (RDW), which is a measure of the variability in size of the circulation erythrocytes. Over the last few years, the association between RDW and various disease outcomes has been studied broadly. Especially the association between RDW and cardiovascular diseases is described in detail. However, little is known with regard to the underlying mechanism for this observed relationship.

In this literature study, I will first give a comprehensive overview of the epidemiology and pathophysiology of atherosclerosis, ischemic heart disease and stroke, as well as their common risk factors. Afterwards I am going to present available publications on RDW and CVD, describe the findings, and discuss possible underlying mechanisms for the observed relationship.

Red Cell Distribution Width (RDW)

Measurement

Red cell distribution width is a measure of the variability in size of the circulation erythrocytes and could be looked at as the electronic equivalent to the anisocytosis judged from a peripheral blood smear. It gives the coefficient of variation of the red blood cell volume in percentage, and thereby expresses the width of the volume curve. At the University Hospital of North Norway, the reference range for RDW is 11.7-14.5. It is calculated by dividing the standard deviation (SD) of the mean corpuscular volume (MCV) by the MCV, and multiplying by 100 to yield a percentage value (figure 1).

$$\boldsymbol{RDW}\ (\%) = \frac{\boldsymbol{SD\ of\ MCV}}{\boldsymbol{MCV}}\ \boldsymbol{x\ 100}$$

(Figure 1)

Most automated blood cell counters calculate this value in a normal blood cell count. This makes it widely available and relatively inexpensive.

What causes a change in RDW?

RDW values vary with diseases that alter the erythropoiesis and the composition of red blood cells. For instance, a condition that increases the release of immature red blood cells from the bone marrow will cause an increase in RDW because the immature cells are larger in volume than the mature cells and thereby leads to a greater span of the volume distribution curve. Hemoglobinopathies, like sickle-cell disease, and other hematological diseases may also cause an increase in RDW (25-27). Traditionally, the measure is used in the differential diagnosis of anemia. Because RDW becomes elevated earlier than other blood parameters, it is also helpful for early diagnosis of nutritional deficiency (28). Folic acid and B12 deficiencies will often present with high values of both MCV and RDW, while an iron deficiency anemia is characterized by a high RDW and low MCV. If blood cells are fragmented, agglutinated or dimorphic, this may also cause an elevation in RDW, and blood samples with elevated levels might need to be examined in a peripheral smear.

Over the last years, RDW has been associated with a vast number of diseases and both cause specific and overall mortality. Associations include conditions affecting almost all parts of the body. The specter is wide, and elevated RDW is associated with outcomes that stretches from mortality in acute pancreatitis (29), mortality in trauma patients (30), risk of dementia (31), mortality in patients with septic shock (32), to an unfavorable lipid profile (33). RDW is also associated to increased HbA1c levels (34), subclinical hypothyroidism (35), mortality in critically ill patients (36), mortality and morbidity in patients with community acquired pneumonia (37), and mortality in patients with chronic obstructive pulmonary disease (38). Lastly, RDW has been described in relation to non-alcoholic fatty liver disease (39), mortality in kidney transplant recipients (40), venous thromboembolism (41), and atrial fibrillation (42). The relation to arterial cardiovascular diseases has also been described in an increasing fashion over the last few years. A more detailed description of these are found under the section on the relation between RDW and CVD. The association to such a wide span of diseases and outcomes has obviously led to questions about the underlying mechanisms for the observed relationships. One thought is that RDW might just be a marker of poor health, and reflects an inflammatory process going on in the body. Inflammatory cytokines are known to influence the bone marrow and erythropoietin, and thereby alter the composition of red cells which leads to a change in RDW. Many studies support this theory. A study including 3845 adult outpatients demonstrated a graded association between quartiles of RDW and high-sensitivity CRP and erythrocyte sedimentation rate. This relationship was independent of hemoglobin levels, MCV, age, sex and ferritin, and was thereby not due to the subjects being anemic (43). The association between RDW and CRP is also described in different selected populations, including overweight adolescents (44), subjects with hypertension (45), and in patients with Alzheimer's disease (46). In 195 patients with systolic heart failure, RDW was found to be associated with an increase in soluble tumor necrosis factor (TNF) receptor I and II. In the same study, multiple correlations were found between RDW and interleukin-6 and C-reactive protein (47). In 144 subjects with adult congenital heart disease, RDW was significantly associated with raised IL-6 levels (48). In patients with non-alcoholic steatohepatitis, RDW was found to be a sensitive and specific method for the assessment of inflammation (49).

A study from 1995 describes the variation in different blood cell parameters throughout a year on an individual level (50). The study included 26 participant which had an 3.4% intraindividual variation of RDW. The interindividual variation in the group was 5.7%.

Erythropoiesis

During a day, the bone marrow of a healthy human produces no less than 10^{12} red blood cells through the process called erythropoiesis. Through formation and maturation of red blood cells, the cells go through several phases in which they have different shapes, sizes and names (51).

It all begins with the stem cell. This passes through the progenitor cells BFU_E (burst-forming unit erythroid) and CFU_{GEMM} (colony forming unit granulocyte, erythroid, monocyte and megakaryocyte) to the first erythrocyte precursor that resembles a red blood cell in the bone marrow, namely the pronormoblast. The pronormoblast, a large cell, goes through several cell divisions to form gradually smaller normoblasts. The cells get smaller, but contain an increasing amount of haemoglobin. The nucleus is eventually squeezed out of the cell, leaving behind some ribosomal RNA which makes it capable of haemoglobin synthesis. The cell is now called a reticulocyte, and is larger than a mature red blood cell. The reticulocyte stays for about 1-2 days in the bone marrow before entering the blood stream. It circulates for 1-2 days losing the rest of its RNA, and shrinking further in size to become a mature erythrocyte. One pronormoblast usually gives rise to 16 mature red blood cells (figure 2) (52).

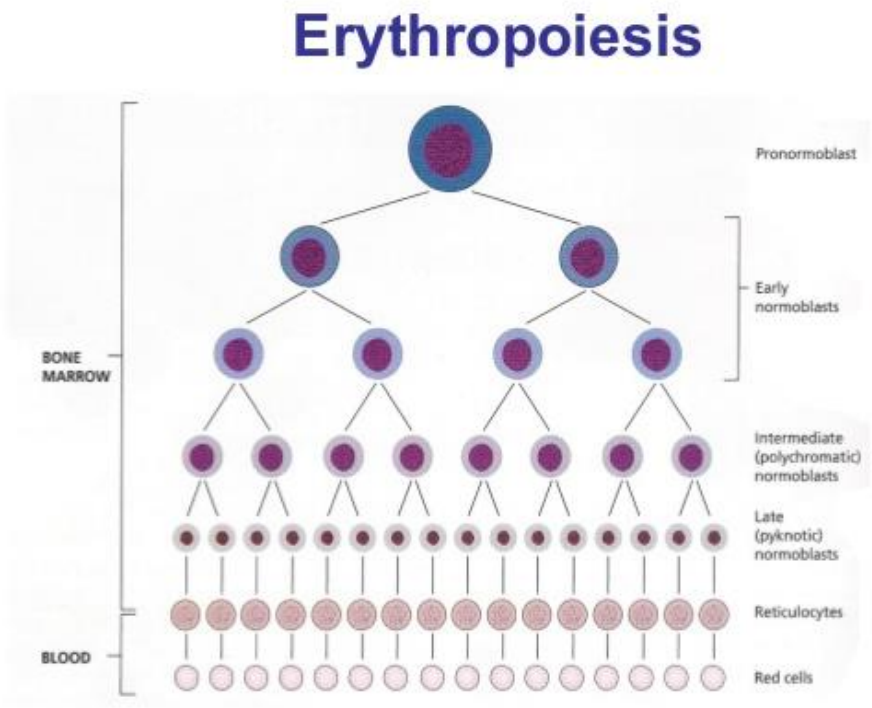


Figure 2, Erythropoiesis, *Essential Haematology, 6th edition*

Either it's through hemolysis, bleeding, anemia or some other condition causing a hypoxic state, the body will respond by producing/releasing erythropoietin (EPO). Normally, about 90% is synthesized in peritubular interstitial cells of the kidneys, and 10% is made in the liver. The hormone is produced in response to oxygen tension in the kidneys. Hypoxia leads toincreased EPO production. EPO is considered the principle regulator of red blood cell production (53). It is crucial for the final step in maturation erythroid cells (54). EPO stimulates many steps in the maturation from stem cell to erythrocyte, including the late BFU_E, the CFU_E, the pronormoblasts and the normoblasts.

The erythropoiesis is an important topic in understanding why the RDW differs both intra- and inter-individually. Everything affecting the synthesis or function of EPO will have some sort of impact on RDW. So will other factors affecting the erythropoiesis. The RDW measures the variation in size of the circulating red blood cells, but does not differentiate between mature erythrocytes and reticulocytes. Thereby, an increased release of reticulocytes will lead to an increased RDW due to the reticulocytes being larger in size than the mature erythrocytes. A reduced release of reticulocytes will have the opposite effect, by increasing the proportion of evenly-sized mature cells. All factors affecting the erythropoiesis, through EPO synthesis, affinity, or through other mechanisms, will have the potential to change RDW.

Arterial Cardiovascular Disease

Atherosclerosis

Epidemiology

Atherosclerosis is the principal cause of heart attack, stroke and gangrene of the extremities (2), and is thereby the underlying disease responsible for some of the leading causes of death world-wide. A study of asymptomatic atherosclerosis in 526 subjects aged 45-84 showed a prevalence increasing rapidly with age. Between 55-64 years of age, 2.4% of males had carotid artery stenosis of any grade. In males aged 75-84, the prevalence increased to 30.3% for stenosis <50% of the lumen, and to 6,1% for stenosis greater than 50% of the lumen. Minimal lesions (< 15%) were very prevalent even at relatively young ages, with 32.1% in males 45-54 years (55). In a study of Finnish men aged 42, 48, 54 and 60 years, only 51% were free of any detectable carotid atherosclerosis. The prevalence in the four age groups were 14.1%, 32.0%, 67.7% and 81.9% respectively (56). Studies show that atherosclerosis starts early. In 262 transplanted hearts with a mean age of 33.2 years, lesions were present in 1 out of 6 (57). For all studies discussed, the prevalence tended to be higher among men than women. In a study of 3016 men and 3404 women aged 25-84 years, both plaque prevalence and morphology differed between the genders. Atherosclerotic plaques were found in 55.4% of men and 45.8% of women. The plaque prevalence was similar in men and women at older ages, with the "turning point" of male predominance at around 50 years suggesting a menopausal effect on the prevalence in women. The men had a higher rate of soft, echolucent plaques than women, maybe explaining why acute coronary events are more common in older men even though the prevalence of atherosclerosis is more or less the same (58).

The hard end points of atherosclerosis in the coronary and carotid arteries, myocardial infarction and stroke, are well known. However, atherosclerosis might cause serious damage all around the body, with potentially critical outcomes. A study exploring renal artery stenosis, an important cause of hypertension and kidney failure, showed that subjects with atherosclerosis elsewhere had a high prevalence of significant renal artery stenosis (59). Peripheral arterial disease (PAD) increases markedly with age. Atherosclerosis in peripheral arteries can lead to claudication and gangrene if they get totally occluded With PAD defined as ankle-brachial index of less than 0.95, the frequency of intermittent claudication increased from 0.6% in subjects 45-54 years, to 2.5% in those aged 55-64 years, and was 8.8% among subjects aging 65-74 years. (60).

Pathophysiology

Through a process called atherogenesis, atherosclerosis is formed in the tunica intima zone of the arterial vessel walls (Figure 3), primarily the large and medium-sized elastic and muscular arteries (3). Normally, this is a very slowly developing lesion that might take decades to become clinically significant. The “atherosclerotic plaques” protrude into the vessel lumen and obstructs the blood flow. This obstruction is dangerous in itself, but the atherosclerotic plaques also weakens the underlying tunica media which may lead to plaque rupture and the creation of an acute arterial thrombosis (61). Such plaque ruptures cause acute myocardial infarctions and strokes.

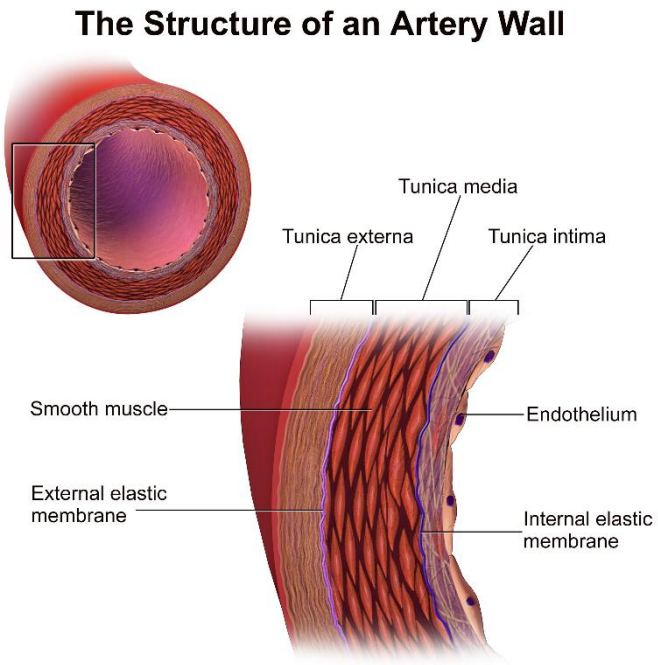


Figure 3, Microscopic anatomy of the artery, *Blausen.com staff. "Blausen gallery 2014"*

Due to the very important role of atherosclerosis in the most deadly diseases in the Western world, it is heavily studied. The current view of the pathogenesis of the disease supports a model that regards atherosclerosis as an inflammatory disease – a chronic inflammatory response from the arterial wall due to endothelial injury (62). The endothelial injury arises from a combination of many different factors. After the primary injury to the vessel wall, the lesion progresses through interactions of modified lipoproteins, monocyte-derived macrophages, T lymphocytes and the normal cellular constituents of the arterial wall, like smooth muscle cells (61). The process of atherogenesis often starts at an early stage in life. Fatty streaks, the earliest type of lesion in the atherogenesis, consist only of monocyte-derived

macrophages and T lymphocytes. Such fatty streaks are common in infants and young children (63). Step by step the atherogenesis can be described like this:

1. Chronic endothelial injury due to a combination of different factors including (but not limited to) hyperlipidemia, hypertension, smoking, homocysteine, hemodynamic factors, toxins, viruses and immune reactions.
    a. The hemodynamic turbulence play an important role in endothelial cell injury, shown by the fact that atherosclerotic plaques tend to form at points where vessels branch out, at ostia of exiting vessels and on the posterior wall of the abdominal aorta, where there are disturbed flow patterns.
    b. The blood pressure plays an important role in weakening the vessel wall. A good example of this is the big difference between the systemic and the pulmonary circulation when it comes to atherosclerosis. The pulmonary artery has a blood pressure far lower than the rest of the body, with an average of about 25/8 mmHg. Partly because of this, atherosclerosis is almost completely absent in the pulmonary circulation (64, 65).
    c. Another important contributor is the hyperlipidemia. It may increase local production of reactive oxygen species leading to accelerated nitric oxide decay, which is important for vasodilatation. This increases the local shear stress on the vessel wall.
    d. Inflammation is also important for injury to the vessel wall. As soon as endothelial cells become dysfunctional, they start expressing adhesion molecules that contribute to leukocyte adhesion. Primarily monocytes and CD4+ T-lymphocytes, but also natural killer T-cells in early lesions. Vascular-cell adhesion molecule 1 (VCAM-1) is typically upregulated in response to hypercholesterolemia, and because monocytes and T-lymphocytes carry the counterreceptors for VCAM-1, these are the blood cells that primarily adhere (66). However, all blood cells passing along the surface of the blood vessel might adhere to the activated endothelium.
2. The injury to the endothelium causes a chronic endothelial dysfunction leading to increased permeability, leukocyte adhesion and emigration. Chemokines in the intima stimulate the blood cells to migrate through the junctions between the endothelial cells, and thereby allows them to pass into the subendothelial space. In the tunica intima, monocytes transform into macrophages and starts eating up lipoproteins like oxidized LDL. This leads to the formation of so-called foam cells. The macrophage

activation leads to cytokine production and further leukocyte adhesions and inflammatory cell recruitment. Cytokines released from the T-cells leads to increased activity in the cytokine cascade and increased production of interleukin-6 and C-reactive protein. Thus, the local inflammatory process of the atherosclerotic lesions leads to a systemic activation. T-lymphocytes in the intima interact with macrophages and generate the chronic inflammatory state (67, 68). At this point the lesion is called a "fatty streak", with primarily intracellular lipids. The activated leukocytes in the atherosclerotic lesion release growth factors that stimulate smooth muscle cell proliferation and synthesis of extracellular matrix. These fatty streaks are prevalent in young people and they never cause symptoms. In some they progress to atherosclerotic plaques, and in some they disappear (69) (Figure 4).

3. Proliferation of smooth muscle cells and deposition of extracellular matrix (collagen) converts the fatty streak into a fully developed atherosclerotic plaque. The center of the atheroma consists of foam cells (macrophages rich on lipids) and extracellular lipid droplets. Around this core there is a cap of smooth muscle cells, and a collagen-rich matrix. Such plaques are relatively stable, but the inflammatory cells in the plaque may produce inflammatory molecules and proteolytic enzymes that induce smooth muscle cell apoptosis and catabolism of the extracellular matrix, which weakens the fibrous cap of the plaque and leads to plaque instability (70).

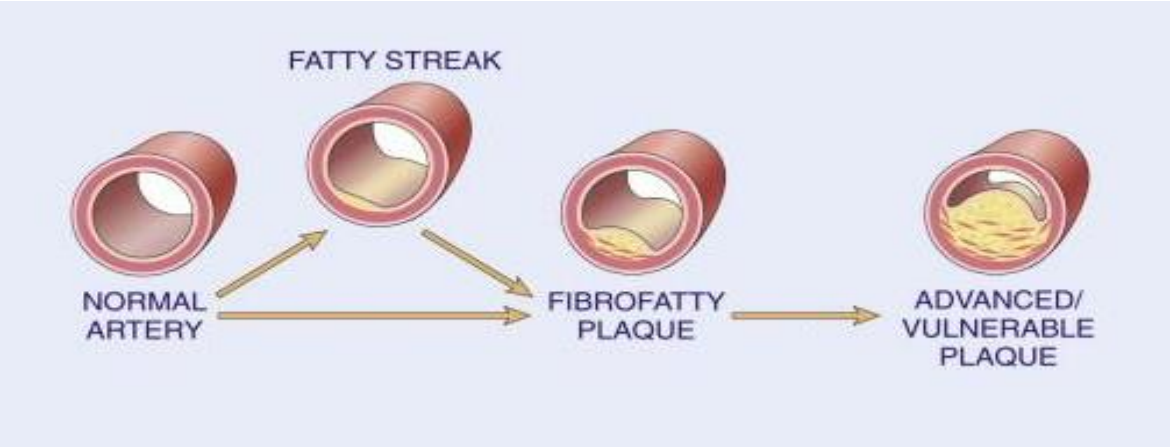


Figure 4, Progression of atherosclerotic plaques, *Robbins basic pathology 8th edition*

Studies show a relationship between atherosclerosis and increased blood plasma levels of inflammatory biomarkers (71-73). Many of the same biomarkers, especially CRP, are associated with angina pectoris, and an increased risk of arterial thrombotic events (74-77).

This relationship is most likely mediated through the ongoing chronic inflammatory process, the atherosclerosis, which precedes the vast majority of these atherothrombotic events.

Ischemic Heart Disease

Ischemic heart disease is a collective term for a group of related syndromes that result from myocardial ischemia. Ischemia occurs when the oxygen demand of the heart muscle, myocardium, is greater than the supply. A situation like this is most often a result of narrowing of the coronary arteries due to atherosclerosis.

Ischemic heart disease presents in its mildest form as angina pectoris. Directly translating to "chest pain", angina pectoris is painful but does not lead to cell death and necrosis of the myocardium. Stable angina pectoris occurs at different levels of exhaustion when the heart rate increases and the oxygen demand becomes too high, for instance during exercise or stressful situations. In the case of stable angina, the pain will disappear after a short while if the person comes to rest. The angina is referred to as unstable if the symptoms occur at progressively less exertion or at rest.

Epidemiology

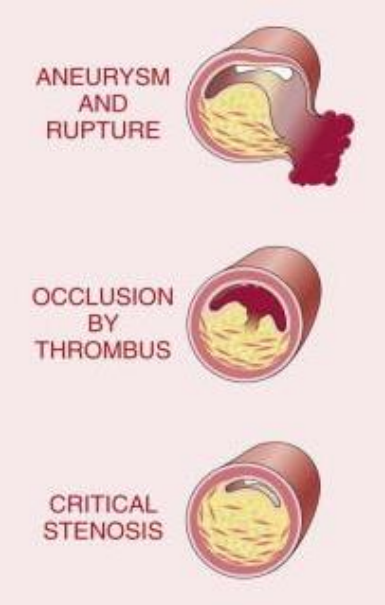
Ischemic heart disease is the leading cause of death both worldwide and in Europe. It is estimated that cardiovascular disease is responsible for over four million deaths yearly in Europe, which makes it attributable for about 51% of deaths in women, and 42% of deaths in men (4, 5). Many of the deaths due to CVD happens prematurely (<75 years), and the disease(s) accounts for 37% of all premature deaths in Europe. According to an update from 2010, the overall prevalence of myocardial infarction in the US population 20 years or older were 4.7% in men and 2.6% in women (78). The prevalence varies with race, especially for men, with a prevalence of 5.1% among non-Hispanic white men, 3.6% among non-Hispanic black men, and 2.6% among Mexican American men. The incidence of fatal and non-fatal myocardial infarctions among women "lagged" around 20 years behind the incidence of men. However, the age-adjusted prevalence of angina pectoris was higher among women than men. The update approximates that about 1 in 6 deaths in the US are caused by coronary heart disease. The incidence and prevalence differs markedly all around the world. In a country like Japan, which is economically comparable to the western world, the rate of coronary heart disease is only one fourth of the rate in North America. This suggests that both genetic variation and life-style play important roles in disease development (79).

Pathophysiology

Myocardial infarction occurs due to prevention of blood flow through the coronary arteries, and is the necrosis of heart muscle cells due to ischemia. A myocardial infarction may be fatal, but it can also be "silent" and go by totally undetected (80). Either by reducing oxygen demand, or by reperfusion through some intervention, the myocardial cells might survive. If not, coronary occlusion will lead to cell death within short time. Most of the myocardial infarctions are a result of coronary thrombosis following thrombus formation on the surface of an activated atherosclerotic plaque (81). These arterial thrombi consists of platelets, fibrin, erythrocytes and degenerating leukocytes. What causes an atherosclerotic plaque to rupture? All the components of the atheroma affect its stability in different ways. Destabilization happens through production of coagulation factors, radicals and vasoactive molecules by the macrophages and T-cells. These molecules inhibit the stable fibrous caps, attack the collagen stabilizing the caps, and initiate thrombus formation (69, 82, 83).

In a typical myocardial infarction, the coronary artery occlusion follows the sequence below, as derived from *Robbins Basic Pathology*:

- It all starts with a sudden break in the surface of an atherosclerotic plaque. This can happen through hemorrhage, ulceration, rupture, or for other reasons. The subendothelial collagen and necrotic plaque contents are exposed to the blood stream.
- Platelets adhere to this disrupted plaque, aggregate, becomes activated, and release potent secondary aggregators including thromboxane A_2, adenosine diphosphate and serotonin.



- Vasospasms are stimulated by mediator release and platelet aggregation.
- Other mediators activate the extrinsic pathway of the coagulation, adding to the bulk of the thrombus.
- It all builds up, and within minutes, the thrombus can have grown so big it completely occludes the lumen of the coronary vessel.
- The atherosclerosis have three main pathogenic events. Aneurysm, occlusion due to rupture of atherosclerotic plaque and thrombus formation, or stenosis due to growing plaque (figure 5).

Figure 5, main pathogenic events of atherosclerosis, *Robbins basic pathology 8th edition*

When the coronary artery blood supply is blocked and the myocardium has to work anaerobically, lactic acid and other breakdown products start accumulating within seconds. Within a minute after onset of ischemia, the heart muscle loses its contractility. However, these changes to the functionality of the heart are reversible. Severe ischemia lasting 20-40 minutes will on the other hand cause necrosis to the myocardium, and permanent damage to the contractile function. Five main factors will determine the size, location and lasting consequences of the myocardial infarction. Severity of the coronary occlusion, size of the area vascularized by the occluded artery, duration of the occlusion, demand by the myocardium, and the extent of collateral supply. With longer blockage than an hour, the microvascular system will also get damaged (61). The tissue with irreversible injury changes over the course of the days, weeks and months following the myocardial infarction. The necrosis following the ischemia – coagulation necrosis – ensues from around the fourth hour after the loss of blood supply and lasts for about three days. During the same period a neutrophilic cells starts infiltrating the tissue. From the third day, the breakdown of dead myofibers starts with some phagocytosis of dead cells by macrophages. Within the first week, phagocytosis of dead cells is at its peak, and some fibrovascular granulation tissue forms at the margins. Between day 10 and 14, granulation tissue is abundant, with new blood vessels and deposition of collagen. The deposition of collagen continues up until about eight weeks after the myocardial infarction, and the cellularity decreases. The scarring process is now complete, and after two months, a dense collagenous scar is visible.

Specific risk factors for myocardial infarction include factors that help cause the atherosclerotic plaque to rupture, and thereby start the whole process of thrombosis formation. The “architecture” of the plaque, the mechanical properties of the blood flow and vessel walls, and the biological composition of the extracellular matrix play a critical role in plaque stability (84). Studies have shown that there is poor correlation between which plaques that rupture and cause myocardial infarctions, and which plaque that are considered “severe” by angiography. In other words – angiography cannot detect the plaques that are vulnerable to rupture (85, 86). This is because angiography says little about plaque structure and biological activity in the plaques core. Furthermore, clinical studies on lipid-lowering treatment show that these drugs lower the risk of cardiovascular events without any changes to the plaque severity as judged by angiography (87). The treatment works on the composition and activity of the plaque, rather than the size and progression.

The walls and atheromas of the arteries undergo a mechanical stress that varies a lot with time. The mean arterial pressure is not constant, and the action of the heart causes a

pulsatile blood pressure leading to a repetitive dynamic impact on the vessel walls. These forces play an important role in plaque rupture, but are more or less the same throughout the arterial system. Which plaques that actually rupture is more dependent on the composition of the plaque core and the strength of the fibrous cap. The weaker the structure is, the less mechanical force (blood pressure) is needed to break it down. In mature atherosclerotic plaques, T lymphocytes and macrophages release proteolytic enzymes and cytokines that weaken the plaque and makes it more prone to rupture. Smooth muscle cells go into apoptosis, while the extracellular matrix is reduced through active degradation and decreased synthesis. The lipid core is also an important component in plaque stability. High levels of cholesterol does not only promote atherogenesis, but is also an important risk factor for rupture of plaques. As clinical trials with lipid-lowering drugs show decreased morbidity and mortality, it is likely to think that a healthier lipid profile changes the composition of the plaque core, and makes it more stable (84).

Stroke

Stroke is a collective term for conditions that lead to an acute loss of blood supply leading to infarction of the brain tissue. This can happen through thrombotic or embolic occlusion of vessels, or rupture of vessels leading to hemorrhage. A global ischemia can also occur in situations with low cerebral perfusion pressure, caused by either a drop in mean arterial pressure, a raise in intracranial pressure, or a combination of the two.

Epidemiology

Cerebrovascular events are the number one cause of permanent disability, and the third most common cause of death in the western world (8-10). Strokes accounted for 1 in every 18 death in the US in 2006. Between ages 45-64 years, 8-12% of ischemic strokes and 37-38% of hemorrhagic strokes are fatal within 30 days (78). Studies show that up to 30% of stroke survivors are permanently disabled, and up to 20% require institutional care within 3 months after onset (88). Even though many survive a first stroke, the prognosis is bad. For patients more than 40 years of age, 47% of men and 51% of women will be dead within five years. Both Japanese and Chinese populations have higher rates of cerebrovascular events than coronary heart events, which is completely opposite from Western populations (79).

Pathophysiology

Most common are the ischemic strokes resulting from a thrombotic or embolic narrowing or occlusion of an artery leading to or within the brain. Contrary to the myocardial infarctions, the ischemic strokes are mostly due to embolic thrombi rather than in situ thrombus formation. Thus, for the majority of the events, the pathophysiological mechanisms leading to the occluded artery are different compared to myocardial infarctions. The emboli leading to focal cerebral ischemia arise from several different sources. As for myocardial infarction, atherosclerosis is the most common source of the thrombus, but for cerebral infarctions to occur the thrombus have to travel a stretch to cause its damage. The carotid bifurcation, the basilar artery and the origin of the middle cerebral artery are frequent sites for atherosclerotic plaque rupture that cause thromboembolism. The thrombus forming on ruptured plaques often fragment into smaller bits that travel with the blood stream and occlude smaller arteries where it is stuck. Ischemic strokes caused by atherosclerosis have essentially the same risk factors as myocardial infarctions (89). In addition to the thrombi caused by atherosclerosis, “mural thrombi” are also common in cerebral infarctions. These are thrombi occurring in the heart

chambers or in the aorta. Previous myocardial infarction, valvular disease, dilated cardiomyopathy, myocarditis, catheter trauma or atrial fibrillation are important factors predisposing for the generation of such mural thrombi (61). Thus, the ischemic strokes due to these mural thrombi have risk factors quite different from the ones previously described (90). The formation of the mural thrombi happens through adherence and aggregation of platelets when the subendothelium/subendocardium is exposed to the blood flow, either in one of the hearts chambers or in the aorta. Seconds to minutes after cerebral ischemia initiates, the "ischemic cascade" starts. Inadequate blood supply leads to anaerobic metabolism and the creation of lactic acid. Lactic acid disturbs the acid-base relationship in the brain cells, and can destroy them through a drop in pH. ATP-dependent ion transporters in the cell walls fail, and the membranes are depolarized. Subsequently, ions are "misplaced", with a large calcium influx and potassium efflux. The excessive calcium entry activates proteases and lipases leading to breakdown of the cells' membrane and the entry of harmful chemicals. The mitochondria break down, and apoptotic factors are released in the cell. Apoptosis starts and the necrotic cells release toxins harming other cells nearby (91).

The hemorrhagic strokes account for about 13% of the stroke cases (92). Hemorrhages within the brain are caused by a variety of reasons. Hypertension or some other cause leading to vascular wall injury is common. Bleeds may also occur due to vascular malformations or brain tumors. Intraparenchymal hemorrhages are often due to the rupture of a small vessel caused by hypertension. Such bleeds typically occur in the basal ganglia, thalamus, pons and cerebellum (61). Subarachnoid hemorrhages, on the other hand, are caused by saccular aneurysms. Such rupture can occur at any time, but are associated with an acute increase in intracranial pressure.

Risk factors for arterial cardiovascular disease

With atherosclerosis being the principal cause of myocardial infarction and stroke, understandably there are many common risk factors for these diseases. Nonetheless, many of the common risk factors have different impact on the different disease outcomes. For instance, it is described that smoking has the largest impact on peripheral arterial disease leading to intermittent claudication, while cholesterol levels has the largest impact on coronary arteries, and blood pressure is the most important factor in cerebral atherosclerosis (64). Atherosclerosis is a multifactorial disease, and the majority of patients have more than one risk factor. It is also important to note that many of the risk factors interact with each other. For instance, blood sugar, cholesterol and hypertension can all be adjusted by changes in diet, physical activity and weight loss.

Not modifiable

The Framingham study shows that the risk of a coronary heart disease increases with age (93). The same study also shows a great difference in incidence between genders. In the study, every fifth man would suffer a coronary heart disease event by the age of 60, while the corresponding proportion is only one in every 17th for women. Family history of a premature coronary heart disease is a strong and independent risk factor for future MI. The risk ratio for MI for subjects with a family history of either parents was 1.61 in men and 1.85 in women (94). Another study showed that a family history of MI was predictive of all-cause and cardiovascular death in men, but not in women (95).

Modifiable

Most of the risk factors for cardiovascular outcomes are modifiable in one way or another, and changes might be achieved through medication and various life-style choices.

Cholesterol has been a well-known risk factor for cardiovascular disease for a long time (21). The 4S study showed that treatment with cholesterol lowering simvastatin in patients with coronary heart disease had clear benefits with regard to both mortality and recurrent coronary events (22). Many similar trials have later shown the same (96-98). Both total cholesterol and LDL cholesterol levels can be used in prediction of CHD risk (99). Cholesterol is an important component of the atherosclerotic lesions as previously described, and high cholesterol levels increases the risk of atherosclerotic development and in turn the risk of myocardial infarction and stroke.

Hypertension plays a key role in both plaque formation and plaque rupture. Through constant pressure on the vessel wall, high blood pressure weakens the endothelium and thereby facilitates plaque formation. Likewise, high pressure on vulnerable plaques increases the risk of rupture (61). Hypertension is very common, increasing in prevalence with age (100), and an important contributor to cardiovascular morbidity and mortality (16, 101-103). Commonly, the goal of antihypertensive treatment has been systolic blood pressure<140 and diastolic blood pressure<90, and <130/<80 for some high risk groups (104, 105). Even in subjects with blood pressure below 140 systolic, there is a substantial potential for cardiovascular risk reduction through further lowering of the blood pressure. In a recent study, 9351 persons were randomized to two different antihypertensive regimes, standard and intensive, with a goal of systolic blood pressure <140 and <120 respectively (106). The study was stopped prior to plan because of a significantly lower risk of morbidity and mortality in the intensive care group.

Both microvascular and macrovascular changes occur in diabetes patients making them more susceptible to a wide range of diseases (107). Strong evidence links atherosclerotic cardiovascular disease with both type 1 and type 2 diabetes (15, 108-110). The vascular changes include endothelial cell dysfunction (111, 112) and vascular smooth muscle dysfunction (113). Individuals with diabetes also have altered more active platelet functions and abnormal coagulation through impaired fibrinolytic capacity (114-116).

Smoking is another well-established easily (?) modifiable risk factor. All the way back in 1938 it was stated that cigarette smoking "impaired survivorship" (117). A summarizing article from 2004 states that cigarette smoking "impacts all phases of atherosclerosis from endothelial dysfunction to acute clinical events". Cigarette smoking increases inflammation, thrombosis and oxidation of low-density lipoprotein (118). Numerous large epidemiological studies have confirmed the dangers of smoking (14, 119, 120). There is also an increased risk of death from ischemic heart disease among passive smokers. Nonsmokers living with smokers have a 30% increased risk of cardiovascular mortality (121).

A large study conducted on youth in three European countries showed that low average activity level was associated with clustering of cardiovascular risk factors (122). Many studies have showed the same (19, 123, 124). Physical activity has a beneficial effect on many of the other cardiovascular risk factors, like hypertension, BMI and blood sugar. However, it also has an independent effect, meaning that overweight, hypertensive and diabetic inactive individuals will be at higher risk than a matched population with a higher activity level.

Many will state that physical activity, diet and overweight go hand in hand, but they are also separate risk factors. A person can be skinny looking, but still "fat" on the inside. The Mediterranean diet is well known for being heart healthy. One would think that a low-fat diet would be the most beneficial for the cholesterol, but a study showed that adding healthy fats through vegetable oils and nuts reduced the cardiovascular risk factors (125).

Alcohol consumption in moderate amounts is shown to have a beneficial effect on cardiovascular disease (24).

Obesity was early described as an independent risk factor for cardiovascular disease, with a dramatically increased disease burden among obese individuals (20, 126).

As previously described, atherosclerosis is considered an inflammatory disease (3, 62), which makes it plausible that the hard outcomes of atherosclerosis, myocardial infarction and stroke, are associated with inflammation. Several studies have described this association. A study from 1997 shows that CRP predicts myocardial infarction and stroke, and states that the reduced risk in individuals using aspirin (acetylsalicylic acid) is due to reduced levels of CRP (76). Two years later, a study concluded that CRP is an independent risk factor for cardiovascular disease, while another described that CRP adds to the predictive value of cholesterol in myocardial infarction (127, 128). Increased CRP also relates to peripheral arterial disease (129). Later, other inflammatory biomarkers have been linked to cardiovascular disease, like interleukin-6 and soluble intercellular adhesion molecule 1 (sICAM-1) (130, 131). Acute systemic inflammation, induced by S typhi vaccine, has been shown to impair endothelium-dependent vascular dilatation in humans, maybe explaining some of the enhanced risk of cardiovascular events in patients with inflammation (132).Monocyte count is shown to be a predictor of novel plaque formation (133).

A study from 2001 concluded that oxidative stress, indicated by a higher response to vitamin C, plays a role in both endothelial dysfunction and in coronary artery disease activity (134). Another study proposes oxidative stress as the pathogenic mechanism that eventually leads to overt diabetes and cardiovascular disease (135).

Atrial fibrillation is a well-established, independent risk factor for stroke (136). In patients with AF, risk of stroke is five-fold increased. The stroke cases attributable to AF increased with increasing age, and was 23.5% for those aged 80-89 years in a study with data from the Framingham study (90). There has been developed different scoring systems for patients with atrial fibrillation to evaluate the need for preventive anticoagulation. One of these is the CHA_2DS_2-VASc score. It incorporates many of the other cardiovascular risk factors listed above, like age, sex, diabetes and hypertension, as well as congestive heart

failure, previous stroke and vascular disease (137). Atrial fibrillation is a modifiable risk factor for many through anti-arrhythmic drugs, electroconversion or ablation.

Relation between Red Cell Distribution Width and Arterial Cardiovascular Disease

Over the last decade, research articles on the relationship between RDW and cardiovascular morbidity and mortality have been published in abundance. For the most part, the results point in the same direction – describing an association between increasing values of RDW and the risk of cardiovascular outcomes and mortality. However, some studies display results showing no association. In this section I will thoroughly go through most of the available literature and discuss different aspects, including methodological, that might explain the observed results. I will primarily focus my attention on the studies I believe to be the strongest, in size and methodology, but also mention the smaller studies. I have obtained the literature from the MEDLINE database by using the search engines PubMed and google scholar. In PubMed I have used medical subheadings (MeSH) to better pinpoint my search. Some of the literature I found in the reference list of other key articles. Search words included, but were not limited to: "RDW, red cell distribution width, erythrocyte indices, blood cell count, red blood cell, risk factor, atherosclerosis, atherosclerotic plaque, peripheral arterial disease, arterial cardiovascular disease, myocardial infarction, heart attack, cerebrovascular events, stroke, ischemic stroke, and all-cause mortality.

RDW and Atherosclerosis

As atherosclerosis is a slowly developing condition, often without any symptoms, it tends to go under the radar. Hard endpoints like myocardial infarction and stroke are easier to quantify and measure, because a person seldom goes through a MI without either dying or going to the hospital. For this reason, the relation between RDW and atherosclerosis is harder to get good data on compared to MI and stroke. Nonetheless, in the effort to describe the mechanisms involved in the relation between RDW and cardiovascular outcomes, the process of atherosclerosis, and how it relates to RDW, is perhaps the most crucial step. The available literature is summarized in table 1, and described in more detail below.

Table 1, overview of available literature on the relationship between RDW and atherosclerosis

First author, journal, year	Study design	Study population	Result/conclusion
Wen, Experimental and clinical cardiology, 2010	Cross-sectional	156 hypertensive patients, 60-85 years	Higher rate of carotid plaque with increasing RDW values
Söderholm, Plos One, 2015	Cohort	5309 with no previous stroke or coronary event	No association between RDW and presence of plaque. Significantly increased IMT in highest RDW quartile.
Lappegård, Thrombosis & haemostasis, 2015	Cohort	4677 subjects	RDW associated with carotid atherosclerosis presence and progresion
Sahin, Medical principles and practice, 2015	Cross-sectional	335 NSTEMI patients divided in two based on SYNTAX score	RDW level independently associated with high SYNTAX score
Chaikriangkrai, North American journal of medical science, 2014	Cross-sectional	868 patients presenting with acute chest pain. No known CAD.	No association between RDW and coronary calcification presence or severity
Khode, Nigerian journal of cardiology, 2014	Case-control	128 subjects (39 with MI, 24 with stable CAD, 65 controls)	No association between RDW and CAD.
Zalawadiya, Vascular medicine, 2012	Cross-sectional	6950 non-institutionalized, nationally representative	Increase in peripheral artery disease with increasing RDW
Lappé, Clinical chimica acta, 2011	Cohort	1489 patients with CAD followed for 8.4-15.2 years	RDW quintiles predicted all-cause mortality in stepwise manner
Su, journal of thoracic disease, 2014	Systematic review	15 studies on patients with known CAD	14/15 studies showed positive relationship between RDW and mortality risk

Sahin, Turk Kardiyol Dern Ars, 2015	Cross-sectional	326 patients with known CAD.	RDW was a predictor of poor coronary collateral circulation
Tanboga, Clinical and applied thrombosis/hemostasis, 2012	Cross-sectional	322 NSTEMI patients	RDW predictor of impaired coronary collateral circulation

A study published in 2010 in *Experimental and Clinical Cardiology* by Y. Wen et al. looked into RDW and risk of carotid atherosclerosis in patients with hypertension. Carotid atherosclerotic plaques were identified using ultrasound in 156 hypertensive inpatients aged 60-85 years. The population was divided into four categories, and the authors found a significantly higher rate of carotid plaque with increasing values of RDW. High baseline RDW was also found to be associated with low hemoglobin levels and a high white blood cell count. This study was conducted on a small population with hypertension, a known risk factor for atherosclerosis, and it could not say anything about the observed relationship independent of anemia (138).

In 2015, Söderholm et al. published a study in *Plos One* regarding RDW and stroke, with some data on the relation between RDW and atherosclerosis as an additional finding. In a cohort of 5309 individuals with no previous stroke or coronary event, the presence of carotid plaque and intima-media thickness (IMT) was assessed by ultrasound. Logistic regression was used to evaluate the relation between sex-specific quartiles of RDW and presence of plaque in the common carotid artery. Linear regression was used to assess the relation between mean IMT and quartiles of RDW. The regression analyses were adjusted for age, systolic and diastolic blood pressure, blood pressure medication, smoking, diabetes, alcohol intake, waist circumference, low physical activity, lipid lowering medication, white blood cell count, history of atrial fibrillation and heart failure, LDL, HDL and triglycerides. In the multivariable adjusted model, the authors did not find any association between RDW quartiles and presence of plaque. There was a significantly increased IMT in the common carotid artery in the highest quartile of RDW (139). This study was conducted in a large sample of previously healthy middle-aged adults, and was well performed. However, it only looked at the prevalence of carotid plaque, and did not follow the individuals over time. The study does not say anything about RDW and formation or progression of atherosclerotic plaques over time.

In 2015 Lappegård et al. published data in *Thrombosis & haemostasis* from the Tromsø study, a cohort with similarities to the population described above. The results were contradictory. A population of 4677 was examined with ultrasonographic imaging of the carotid artery at two separate occasions 7-8 years apart. Subjects were divided into tertiles depending on RDW level. The mean change in total plaque area increased significantly with increasing RDW value in multivariable analyses, both with RDW modeled as a categorical and continuous variable (140). The adjustment model included many common cardiovascular risk factors including hs-CRP levels.

The following three studies have looked into the relationship between RDW and the presence and severity of coronary artery disease. Two of them found no relationship (141, 142), whereas the last one did (143) Positive news first. Sahin et al. published a study in *Medical Principles and Practice* on the association between RDW and the severity of coronary artery disease in patients with NSTEMI. The severity of CAD was assessed with a scoring system called SYNTAX. This scoring system gives "points" based on number of lesions, amount of myocardium distal to lesion and morphological features of the lesions based on coronary angiography. A total of 335 patients with NSTEMI were enrolled in the study. The authors divided the population in two based on the SYNTAX score, and found that RDW levels were independently associated with a high score (141). They did not find any association between RDW and long-term mortality in the NSTEMI patients. The obvious weakness to this study is the population consisting of patients with a previous NSTEMI. To determine whether the RDW increased because of the severity of the carotid plaque or vice versa is hard because they all had plaque. In my discussion regarding the mechanisms for the association between RDW and MI/stroke, this study adds little, as all the subjects already had an MI and it's hard to tell whether the increased RDW was a cause or a consequence of the MI.

Chaikriangkrai et al. published contradictory results in the *North American Journal of Medical Science* in 2014, where 868 consecutive patients presenting with acute chest pain were enrolled. The patients did not have known CAD. The patients went through a multi-detector cardiac computed tomography scan, and a coronary artery calcium (CAC) score was given to rate the CAD. RDW and other clinical characteristics were compared among different CAC groups. The researchers did not find a statistically significant association between coronary calcification presence or severity, and the hematological indices including RDW, MCV and hemoglobin (142).

Another study on RDW and CAD published in the *Nigerian Journal of Cardiology* by Khode et al., and reported no association between RDW and CAD (143). However, the authors does not say anything about how the CAD was assessed, and it is therefore hard to interpret the results.

Zalawadiya et al. (144)looked at RDW and peripheral artery disease (PAD) in a study published in *Vascular Medicine* in 2012. It was a cross-sectional study of 6950 participants in the National Health and Nutrition Examination Survey. Peripheral artery disease was defined as an ankle-brachial index below 0.9, and 618 of the participants had such values. With increasing quartiles of RDW, a graded increase in prevalent PAD was observed. 4.2% in the lowest RDW quartile had PAD, while 13.9% in the highest quartile did. In analysis adjusted for age, sex, race, body mass index, hypertension, hyperlipidemia, diabetes, smoking, estimated glomerular filtration rate, C-reactive protein, hemoglobin, mean corpuscular volume and nutritional factors, they found a significantly increased odds (OR) of 1.19 (95% CI 1.06-1.34) with each unit (0.1) increase in RDW. The study population consisted of non-institutionalized, nationally representative individuals.

Though the literature on RDW and the formation and progression of atherosclerotic plaques is limited, several studies look into how RDW relates to morbidity and mortality in patients already known to have CAD. A study published in *Clinical Chimica Acta* in 2011 by Lappé et al. looked into 1489 patients with CAD followed for 8.4-15.2 years. The relation between RDW and all-cause mortality was studied using Cox regression. I addition, 449 patients without CAD were evaluated in the same way. The authors found that RDW, when divided into quintiles, predicted all-cause mortality in a stepwise manner, with a multivariable adjusted hazard ratio (HR) of 1.21 (95% CI 1.13-1.29) per quintile increase. An even stronger relationship was found in the group without CAD where the HR for all-cause mortality was 1.33 (95% CI 1.15-1.55) per quintile (145). As there are speculations to whether the link between RDW and inflammation is the mechanism behind the associations, the study would benefit from using controls that were recruited from a healthy population, and not other patients, which might suffer from something just as deadly as coronary artery disease.
In 2014 Su et al. published a systematic review and meta-analysis in the *Journal of Thoracic Disease* looking into the relation between RDW and risk of cardiovascular events, and mortality, in patients known with CAD. 15 studies were included in the meta-analysis, with only one reporting a negative association (146). The size and quality of the studies varied a lot. Study populations ranged from 100-29526 and mean follow-up time from 1 month to 23 years. Nine of the studies looked at all-cause mortality in CAD-patients. The summary

estimate from these studies, comparing the highest RDW category with the lowest, gave a pooled risk ratio of 2.20 (95% CI 1.42-3.39). The meta-analysis also indicated a significantly increased risk of fatal CVD events (four of the studies investigated this). The pooled RR was 1.80 (95% CI 1.35-2.41). Eight of the studies looked at non-fatal CVD and gave a pooled estimate of 1.86 (95% CI 1.50-2.31). The heterogeneity between the included studies varied due to difference in RDW assays, study populations, outcome definitions and how well they adjusted for potential confounders. The analysis looking into all-cause mortality had a high heterogeneity with I^2=93%, while it was 44% for the study of fatal CVD events, and 28% for the non-fatal CVD events. I^2 says something about the percentage of variance in the analysis that is attributable to the study heterogeneity (147).

In one retrospective study Şahin et al. looked into how RDW correlates with coronary collateral circulation in patients with known CAD. Collateral flow was graded using the Rentrop classification in 326 patients. The population was divided into two groups depending on their collateral circulation (good: rentrop score 2-3, poor: 0-1). The total number of vessels with >95% stenosis did not differ between the groups. Neither did the Gensini scores. The study population was evenly divided, with 155 subjects having good collateral flow, and 171 bad. Multivariate logistic regression was used, and the adjustment model included neutrophil/lymphocyte ratio, MPV, fasting glucose, gamma glutamyl transferase, uric acid, and LDL cholesterol. RDW was found to be a predictor of poor coronary collateral circulation, with OR of 1.73 (95% CI 1.30-2.29). The authors also found that the proportion of subjects with RDW values above the normal limit (14.5% in this study), was significantly higher in the group with poor collateral flow (148). All of the patients in this study had CAD, and no significant difference in severity was found between the groups. This strengthens the theory that the increased RDW might affect the collateral flow directly and is not just due to a more severe atherosclerotic disease. Tanboga et al. investigated the same in a population of NSTEMI patients instead of patients with CAD. The results were similar, with RDW (OR 1.52, 95% CI 1.30-1.78) found to be an independent predictor of impaired coronary collateral circulation (149).

The literature is relatively consistent in the results regarding the relationship between RDW and atherosclerosis. Defined as either carotid atherosclerosis, peripheral artery disease or coronary artery disease, most studies find a positive association between the two. However, there are some studies reporting otherwise. The studies vary a lot in quality due to parameters such as study populations, methods, and ability to adjust for confounding factors .Only one of

the studies above is conducted in a prospective fashion. This means it is hard to tell whether the disease or the increased RDW came first.

RDW and Myocardial Infarction

As opposed to the data on RDW and atherosclerosis, there are more high-quality prospective studies available on the relationship between RDW and MI, as summarized in table 2. Some studies also describe the predictive value of RDW in subjects with established heart disease, these will be discussed as well.

Table 2, overview of available literature on the relationship between RDW and MI

First author, journal, year	**Study design**	**Study population**	**Result/conclusion**
Tonelli, Circulation, 2008	Post hoc analysis	4111 participants in CARE followed for median 59,7 months	Top RDW quartile had 56% increased risk of fatal coronary disease or non-fatal MI
Zalawadiya, The American journal of cardiology, 2010	Cross-sectional	7556 healthy subjects from NHANES	RDW associated with increasing Framingham risk of coronary event
Li, Experimental and therapeutic medicine, 2015	Cross-sectional	392 patients with known coronary artery disease	RDW associated elevated Framingham risk of coronary event
Borné, Heart, 2014	Cohort	26820 subjects without prior MI followed for a mean 14 years	RDW significantly associated with all coronary events and fatal coronary events, but not with non-fatal events.
Arbel, Thrombosis & haemostasis, 2013	Registry-based, retrospective cohort	225006 subjects from health registry, mean follow-up 5,23 years	No association between RDW and increased risk of cardiovascular disease in men, slight increase in women.
Chen, American journal of epidemiology, 2010	Cohort	3226 with no history of cardiovascular disease followed for median 15,9 years	No association between RDW quartiles and coronary heart disease event
Skjelbakken, Journal of the American heart association, 2014	Cohort	25612 participants with no previous MI followed for median 15,8 years	Top RDW quintile had 34% increased risk of incident MI

Lee, Clinical cardiology, 2013	Cohort	1596 patients with MI followed for up to 12 months	Markedly increased risk of second fatal/non-fatal MI with increasing RDW
Sun, Cardiology, 2014	Cohort	691 STEMI patients followed for mean 41,8 moths	Increased risk of all-cause mortality in top RDW group.
Gul, Coronary artery disease, 2012	Cohort	310 NSTEMI/unstable angina followed for up to 3 years	Increased mortality rate with increasing RDW
Azab, Cardiology, 2011	Cohort	619 NSTEMI patients followed after discharge	Increased mortality rate with increasing RDW
Poludasu, Thrombosis & haemostasis, 2009	Cohort	859 PCI patients followed for median time of four years	High RDW a strong predictor of mortality
Uyarel, Coronary artery disease, 2011	Cohort	2506 STEMI patients undergoing PCI followed for median 21 months	High RDW group associated with in-hospital death, and death after discharge

A study published in *Circulation* in 2008 by Tonelli et al. was among the first larger studies to investigate the association between RDW and myocardial infarction. It was a post hoc analysis of the Cholesterol and Recurrent Event study (CARE), a randomized trial of pravastatin vs placebo in subjects with previous myocardial infarction. RDW measurements was obtained from 4111 participants, which were followed for a median of 59.7 months. Cox hazard models were used to analyze the relationship between baseline RDW and different cardiovascular outcomes. In a comprehensive adjustment model, subjects in the top RDW quartile had a 56% increased risk of fatal coronary disease or non-fatal myocardial infarction, when compared to subjects in the lowest quartile (HR 1.56, 95% CI 1.17-2.08) (150). The full adjustment model included age, sex, various cardiovascular risk factors, medications and kidney function. A clear weakness of this study is the fact that all participants have already suffered a myocardial infarction upon enrollment. This means that all subjects have an intrinsic risk of a recurrent event, and the generalizability of the results is difficult. No repeat measurements of RDW was carried out, so we have to rely on baseline values, and expect that they stay about the same for a median of 5 years.

A study published in *The American Journal of Cardiology* by Zalawadiya et al. (151) evaluated the effect of RDW on coronary heart disease events in 7556 multiethnic, healthy and nationally representative subjects recruited from the National Health and Nutrition

Examination Surveys (NHANES). Based on the Framingham risk of coronary events, the population was divided into three groups, with <10%, 10-20%, or >20% increased 10-year risk of a hard coronary heart disease event. The authors found that for every 0.1 increment in RDW, there was a significantly increased odds of being in the Framingham middle (OR 1.35 95% CI 1.27-1.45) or high-risk group (OR 1.38 95% CI 1.25-1.53). This study does not really say anything concrete about how high RDW values relate to the risk of future myocardial infarctions, because it lacks hard end points. It illustrates how RDW correlates to the variables included in the Framingham risk assessment, like blood pressure, cholesterol, smoking and age.

A study by Li et al. (152) had a similar approach when they evaluated the relation between RDW and the Framingham risk score in patients with known coronary artery disease. The study material consisted of 392 patients undergoing coronary angiography. In a multivariable linear regression model they found an association between higher levels of RDW and an elevated Framingham risk score.

In 2014 Borné et al. (153) published data from a large cohort study in *Heart* showing a relationship between RDW and incidence of coronary events. A total of 26820 study participants in the Malmö Diet and Cancer study without prior myocardial infarction or stroke had RDW measured at baseline, and were followed for a mean of 14 years. During follow-up, 1995 participants had a non-fatal or fatal coronary event. Subjects were divided into sex-specific quartiles of RDW. Cox regression models were used to assess risk of coronary events across quartiles of RDW. An acute coronary event was defined as either a hospital diagnosis of acute myocardial infarction, or death due to ischemic heart disease. In adjusted analyses, baseline RDW was significantly associated with risk of fatal coronary events (HR 1.82, 95% CI 1.35-2.44), but not with non-fatal coronary events (HR 0.96, 95% CI 0.82-1.12). In this study, a fatal coronary event was defined as death within 28 days after the occurrence. As RDW is highly associated to anemia, all anemic subjects (n=826) were excluded, and the same analyses were run. The risk estimates remained more or less the same in the non-anemic population (HR1.81, 95% CI 1.35-2.45). The authors found a significant interaction between RDW and smoking, leading to analyses stratified for smoking status. Among non-smokers, the risk of a fatal coronary event was attenuated compared to the analyses in the whole population, but still significant with a 48% increased risk (HR 1.48, 95 CI 1.04-2.10) when comparing the fourth and first RDW quartile. As for many of the other studies, RDW was only measured at baseline, leaving little knowledge about how changes in RDW affects risk estimates over time.

Comparable to the study discussed above, Arbel et al. (154) found no association between RDW and increased risk of cardiovascular events in men, and a slightly increased risk in women, in a registry-based, retrospective cohort study of 225006 subjects. The study describes a relation between RDW and all-cause mortality, but says nothing about cardiovascular mortality. Subjects with RDW >17% were compared to subjects with RDW <13%. Over a mean follow-up of 5.2 years, the HR of a major cardiovascular event was 1.08 (95% CI 0.82-1.41) for males, and 1.26 (95% CI 1.03-1.52) for females. The multivariable model included adjustments for age, hemoglobin, blood glucose, HDL, triglycerides, BMI, diabetes, hypertension, smoking and COPD. In analyses of all-cause mortality they found a strong association with increasing RDW. Again comparing RDW >17% to RDW <13%, the authors found that men had a HR of 4.57 (95% CI 3.35-6.24), and women a HR of 3.26 (95% CI 2.49-4.28) for all-cause mortality. After exclusion of anemic subjects, the association was strengthened. This study boasts some strong numbers with 225006 participants, and 21939 cases of incident major cardiovascular events. However, the study participants are not representative for the general healthy population. They are all above the age of 40, and selected from a health register where all participants had their blood drawn sometime within the last year upon inclusion. In other words, they were all at the hospital for some reason, and probably have some intercurrent illness. Another big issue is the broad definition of a major cardiovascular event in this study, as it includes patients with ischemic heart disease, chronic heart failure, peripheral vascular disease, cerebrovascular event, transient ischemic attack, or patients that underwent percutaneous coronary intervention or coronary artery bypass surgery. All in all, there can't be drawn any specific conclusions about RDWs impact on the risk of myocardial infarction from this study.

In a community cohort in Taiwan, RDW was measured in 3226 individuals with no history of cardiovascular disease (155). The study participants were followed for a median time of 15.9 years. Chen et al. published findings from this cohort regarding RDW and risk of coronary disease in the *American Journal of Epidemiology* in 2010. In this study the term coronary heart disease included study participants with a nonfatal myocardial infarction, fatal coronary heart disease, or participants that were hospitalized for percutaneous coronary intervention or coronary bypass surgery. Subjects were divided into quartiles according to their RDW values, and Cox proportional hazard models were used to evaluate the associations between baseline RDW and risk of future cardiovascular disease or death. During follow-up, 151 participants developed coronary heart disease. The crude analyses did not yield convincing evidence with a HR of 1.16 (95% CI 0.76-1.78) when comparing the top quartile

(RDW >13.7%) with the bottom quartile (RDW ≤ 12.7%). The association was further weakened after adjustments for confounding variables, with a HR of 1.05 (95% CI 0.65-1.68). Again, this was not a study looking specifically into myocardial infarctions.

In a study by Skjelbakken et al. (156) the specific association between RDW and myocardial infarction was studied in a large cohort with subjects recruited from the general population. During a median follow-up of 15.8 years, 1779 of the 25612 study participants suffered a first-ever myocardial infarction. In categorical analyses, the bottom RDW quintile was used as the reference group. In a multivariable model adjusted for common cardiovascular risk factors, subjects in the top quintile had 34% increased risk of an incident myocardial infarction (HR 1.34, 95% CI 1.11-1.60). The risk estimate was further increased to 71% (HR 1.71, 95% CI 1.34-2.20) when subjects with RDW values above the 95th percentile were compared to those in the bottom quintile. In continuous analyses, a 1% increase in RDW yielded a 16% increased risk of myocardial infarction (HR 1.16, 95% CI 1.11-1.21). Analyses were stratified for smoking status, this adjustment increased the risk estimate among smokers while decreasing it among non-smokers. Risk estimates remained essentially the same after exclusion of anemic subjects. Like all the other studies to date, this study did not have repeated measurements of RDW.

Several studies have investigated the value of RDW as a prognostic marker in myocardial infarction patients. Lee et al. (157) investigated the association between RDW and major adverse cardiac events (MACE) in patients with previous myocardial infarction. Patients with MI were followed for up to 12 months with MACE endpoints being either death or a second nonfatal MI. In total, 1596 patients were divided into quartiles depending on their admission RDW value. In Cox proportional hazard models adjusting for confounding variables the HR for MACE when comparing the top with the bottom RDW quartile was 6.18 (95% CI 2.10-18.21) (157).

Sun et al. (158) followed 691 STEMI patients free of heart failure for a mean time of 41.8 months. The participants were divided into two groups depending on RDW value. Multivariable analyses showed that subjects in the top group had a hazard ratio of 3.43 (95% CI 1.17-8.32) for all-cause mortality when compared to the bottom group (158). The study had a low number of events, with only 47 patients dying during follow-up.

A study by Mehmet Gul et al. (159) followed 310 patients with NSTEMI and unstable angina for up to 3 years after discharge. Participants were divided into tertiles depending on RDW value. In Kaplan-Meier survival analysis the cumulative mortality rate during 3 years after hospital discharge was 5.6% in the lowest RDW, and 19% in the top tertile.

A similar study by Azab et al. (160) followed 619 NSTEMI patients after discharge. After adjustments for confounding variables they found that patients in the top RDW tertile had a 30% 4-year mortality rate vs 7% in the bottom tertile (160). Each unit increase in RDW yielded a 10% increased risk of 4-year all-cause mortality (HR 1.10, 95% CI 1.004-1.213).

Two studies have described the prognostic value of RDW in patients admitted for percutaneous coronary intervention (PCI). In a study by Poludasu et al. (161) published in *Thrombosis and Haemostasis* in 2009, 859 PCI patients were followed for a median time of four years and 95 died. In multivariate analyses, high RDW was found to be a strong predictor of mortality in patients with hemoglobin >10.4, but not in those with Hb <10.4. The authors argue that severe anemia in itself is a strong predictor of adverse outcome in PCI patients, and therefore the predictive power of RDW is reduced in this subgroup. The other comparable study by Uyarel et al. (162) followed 2506 STEMI patients undergoing PCI for a median time of 21 months. The study population was divided into a high and a low RDW group. High RDW was associated with both in-hospital death, and death after discharge from the hospital.

To sum up, the evidence is contradictory with regard to the relation between RDW and incident myocardial infarction. Large population-based cohort studies have shown varying results. One large cohort studies did not find any association between RDW and future coronary event, while one found borderline evidence, and one presented definite evidence. There were some important differences in the definition of cases between the three studies. In the study by Chen et al. from the Taiwan cohort, a coronary event was widely defined and included clinically stable patients admitted for coronary artery bypass surgery or percutaneous coronary intervention. Even with the wide definition, the case numbers were far weaker than in the other two studies. In the study by Borné et al. the events only included acute myocardial infarctions, but they were defined as "fatal" if a subject died within the first 28 days after the event. In analyses of non-fatal events, they found no association, but in analyses of all the events put together, the risk estimates showed an association. Those results are more comparable to the study by Skjelbakken et al. which also looked at fatal/non-fatal myocardial infarctions combined, and reported a clear association between RDW and events. The latter study has a more generalizable study population compared to the study by Borné, and a more precise definition of outcome event compared to the study by Chen. Based on the studies presented I believe it's reasonable to conclude that there is a clear association between RDW and myocardial infarctions in western world populations. The disease distribution is very different in East Asia, and might some of the explanation for the varying results presented above.

Studies investigating all-cause mortality in patients with heart disease, all point in the direction of a predictive value of RDW.

RDW and Stroke

The available evidence on the relationship between RDW and stroke is summarized in table 3.

Table 3, overview of available literature on the relationship between RDW and Stroke

First author, journal, year	Study design	Study population	Result/conclusion
Tonelli, Circulation, 2008	Post hoc analysis	4111 participants in CARE followed for median 59,7 months	Top RDW quartile HR of 2.58 for stroke compared to bottom
Söderholm, Plos One, 2015	Cohort	26879 previously healthy subjects followed for median 15,2 years	Top RDW quartile had a 31% increased risk of stroke compared to bottom
Lappegård, Thrombosis & haemostasis, 2015	Cohort	25992 subjects from the general population. Median follow up 15,8 years	Subjects in top RDW quintile had a 37% higher risk of incident stroke compared to bottom
Ramírez-Moreno, International journal of stroke, 2013	Case-control	224 incident ischemic stroke, 224 controls	Dose-response relationship between RDW and risk of stroke
Kaya, Clinical and applied thrombosis/haemostasis, 2013	Cohort	133 heart failure patients followed for 1 year, 14 stroke events	RDW above 15,2% predicted stroke with 87% sensitivity and 74% specificity
Saliba, The American journal of medicine, 2014	Registry based	41140 Subjects with atrial fibrillation	Association between RDW and stroke

The previously described study by Tonneli et al. additionally investigated the relationship between RDW and stroke (150). In fully adjusted analyses, subjects in the top RDW quartile had a hazard ratio for stroke of 2.58 (95% CI 1.47-4.55) when compared to the bottom quartile. As discussed above, this study was not conducted in a healthy population, but in CARE study participants with a previous myocardial infarction.

Söderholm et al. (139) published a study on RDW and atherosclerosis, but the primary focus on the study was stroke. This large (n=26879), population-based cohort of previously healthy subjects provides the strongest evidence on the relation between RDW and stroke thus far. The population was divided into quartiles of RDW and followed for a median time of 15.2 years. During follow-up 1869 participants suffered a first ever stroke. Compared to the

reference bottom quartile, subjects in the top quartile had a 31% increased risk of total stroke (HR 1.31, 95% CI 1.11-1.54) in a multivariable adjusted model. The results were essentially the same in analyses looking specifically into cerebral infarctions (n=1544). The association was borderline significant for intracerebral hemorrhage (HR 1.44, 95% CI 0.90-2.30), while there was no association between RDW values and subarachnoid hemorrhage (HR 0.94, 95% CI 0.43-2.07). A limited number of events, and thereby low statistical power, might explain this, as only 75 subjects suffered a subarachnoid hemorrhage during follow-up.

Lappegård et al. published very similar results in *Thrombosis & haemostasis (163).* The cohort consisted of 25992 subjects recruited from a general population, aging from 25 years and up. During a median follow-up of 15.8 years, 1152 participants experienced a first-ever stroke. When comparing top with bottom quintiles in a multivariable adjusted model, subjects with the highest RDW values had a 37% increased risk of stroke. Subjects in the top 95th percentile had a 55% increased risk. The risk estimates remained the same after exclusion of subjects with anemia. There was no association between RDW and hemorrhagic strokes in subtype analyses, but these analyses were limited by weak numbers. RDW was not associated with risk of all-cause mortality within one year or during the entire follow-up, after incident stroke.

A case-control study by Ramírez-Moreno et al. (164) investigated 224 incident ischemic stroke events and 224 randomly selected controls from the same source population. The population was divided into RDW quartiles, using the lowest quartile as a reference group. Subjects in the fourth quartile had an OR of 5.9 (95% CI 3.1-11.4). The authors also found a continuous dose-response relationship between RDW and risk of stroke.

Kaya et al. (165) followed 133 patients with heart failure (NYHA class 1-3) for 1 year until 14 of them developed a stroke. Receiver-operating characteristics (ROC) curves were used to estimate sensitivity and specificity of RDW for predicting stroke. They found that RDW above or equal to 15.2% on admission had a 87% sensitivity and a 74% specificity in predicting stroke. An association between RDW and stroke was also described in a registry based study with subjects with atrial fibrillation (166), while a large cohort study found a relationship between RDW and atrial fibrillation (167).

All the studies presented show a positive relationship between RDW and risk of stroke events. In the studies looking into subtypes of stroke events, there were no associations between RDW and risk of hemorrhagic stroke events.

Discussion

Underlying mechanisms for the observed association

While numerous studies show relationships between RDW and various disease outcomes, none have been able to conclude why we observe these associations. Does increased RDW have a direct impact on pathophysiological mechanisms, or is it merely a marker of disease, reflecting something else going on in the body?

RDW as a direct influence on disease mechanism

Increased RDW reduces red cell deformability, which in turn can impair blood flow in the microcirculation, leading to hypoxia and increase risk of cardiovascular disease events (168). Such hypoxia might also stimulate to the release of EPO and thereby have an effect on erythropoiesis. A study from 1984 concludes that both red cell deformability and red cell size plays a role in how platelets interact with the vessel wall, and thereby affects thrombus formation (169).

Erythrocytes contain a substantial amount of cholesterol in their membranes, which contributes in the progression of atherosclerotic plaques (170). In addition to adding on as building blocks, the accumulation of free cholesterol within the atherosclerotic plaque makes it more vulnerable to rupture (171). The erythrocyte membrane is regarded as an important source of this cholesterol (172, 173). Cholesterol content in erythrocyte membranes has been described as a novel biomarker of clinical instability in coronary artery disease, and increased RDW has been associated to this increased cholesterol content (174). This might be one way to explain both the association between RDW and atherosclerosis formation/progression, and it's relation to myocardial infarctions and stroke.

RDW as a marker of some other underlying cause

Since RDW is a numeric concept with its value calculated from something else (MCV), and not something that can be measure directly, one can suppose that its relationship to disease must be mediated by other factors. This theory is supported by the fact that RDW is associated with such broad specter of disease. It is unreasonable to think that RDW has a distinct impact on all pathophysiological mechanisms in diseases ranging from dementia to myocardial infarction, but rather describes some sort of disease presence.

Numerous studies on populations with various conditions/diseases show a relationship between RDW and risk of mortality. This supports the theory that RDW reflects an

underlying disease process going on in the body, and that this underlying condition makes the subject more vulnerable. One way to assess such an "underlying disease" is through inflammatory markers. As described above, both RDW and cardiovascular disease outcomes are heavily associated with several markers of inflammation, including CRP, white blood cell count and interleukins. In contradiction, studies have also shown that RDW is associated to disease outcome and mortality after adjustments for inflammatory markers. An association between RDW and mortality was seen in a selected population with normal CRP and white blood cell count levels (175).

In the CARE study (176), there was an association between RDW and myocardial infarction. Subjects in the top RDW quartile had a 56% increased risk of fatal or non-fatal myocardial infarction. In the same population, analyses of the relation between CRP and myocardial infarction was conducted, with very similar results. Subjects in the highest CRP quintile had a 77% higher relative risk of a recurrent coronary event. The relative risks were matched for age and sex, but not adjusted for any other confounding variables.
If the link between RDW and cardiovascular disease were to be explained by inflammation, how might inflammation alter RDW levels? It is stated that interleukin-6 stimulates erythropoiesis (177). The same has been shown for IL-12 (178). Stimulation of erythropoiesis will lead to release of more immature erythrocytes into the blood stream. These are larger in size and will thereby increase the RDW.

On the other hand, studies also describe an inhibiting effect on the erythropoiesis induced by inflammatory cytokines TNF-alfa, IFN-gamma (179). Pro-inflammatory cytokines cause a deficiency in erythropoietin and thereby mediates anemia. Whether such a situation might lead to an increase in RDW, is uncertain. The oldest, smallest erythrocytes will in time die, leading to a shift in size distribution. This might actually cause a reduction in RDW by further evening out the size of the remaining erythrocytes. Another option is that the bone marrow tries to compensate for the anemic situation by prematurely releasing immature, large, blood cells (reticulocytes) due to increased erythropoietin release following the anemic state, which in turn will increase the RDW. The anemic state has a stimulating effect on EPO production which might suppress the apparent inhibiting effect of the pro-inflammatory cytokines.

Increased levels of reactive oxygen species (ROS), leads to ineffective erythropoiesis (180). Uncontrolled increase in ROS can lead to hemolysis and in turn an increase in RDW by the mechanisms described above (181).

The clinical use of RDW

Many theories exist regarding the reason why RDW is associated with such a wide specter of disease. In the literature, a *risk factor* for a disease outcome is described as a factor that precedes the disease, predicts it, and that is directly involved in the biological causal pathway of the disease (182). A *biomarker*, on the other hand, is a biological indicator of some process involved in the disease development. Based on this definition, there are doubts to the whether the variation in size of red blood cells is a risk factor in any of the diseases described above. However, the literature more or less agrees in purposing RDW as a marker of some underlying disease/inflammatory state. With inflammation playing a key role in atherogenesis, this makes it reasonable to call RDW a biomarker for the diseases described above. This way, the use of RDW in risk stratification has a true potential. RDW has been shown valuable in prediction of both first time events and recurrent events/mortality. For instance, RDW could be implemented in established risk calculators for disease outcome, severity, prognosis etc. As a biomarker it has great potential value because it is independent of many well known risk factors, and could thereby add new information. As it is inexpensive and readily available, it might be a variable that clinicians should pay more attention to, and more often include in the blood works of their patients. Nonetheless, one must explore and clarify the underlying mechanisms for the observed relationships in order to introduce targeted preventive treatment in patients with high RDW.

Conclusion

RDW is an inexpensive lab value readily available from a standard blood cell count. It is related to atherosclerosis, myocardial infarction and stroke. In this literature study I have described the basic epidemiology and pathophysiology of these disease entities, and afterwards presented and discussed available research on the topic. This all gave a comprehensive background for the discussion on why we see these relationships between RDW and cardiovascular disease. The underlying mechanism for the associations remains unclear, but it is heavily argued that RDW is a marker of some underlying disease state/inflammatory state. Thereby, RDW is a potential novel biomarker for risk stratification and prevention of disease.

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