

Red Cell Distribution Width is associated with future risk of incident stroke. The Tromsø Study

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Running title: RDW and stroke

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Extra table

What is known on this topic	What this paper adds
<ul style="list-style-type: none">- Recent studies have suggested a relationship between RDW and stroke- RDW is associated with cardiovascular mortality and all-cause mortality	<ul style="list-style-type: none">- RDW is associated with risk of incident stroke independent of anaemia, in a population-based cohort

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Summary

Red cell distribution width (RDW), a measure of the variability in size of the circulating erythrocytes, is associated with cardiovascular morbidity and mortality. We aimed to investigate whether RDW was associated with incident stroke and case fatality in subjects recruited from the general population.

Baseline characteristics were obtained from 25992 subjects participating in the fourth survey of the Tromsø Study, conducted in 1994/95. Incident stroke was registered from inclusion until December 31st 2010. Cox regression models were used to calculate hazard ratios (HR) with 95% Confidence Intervals (95% CI) for stroke, adjusted for age, sex, body mass index, smoking, haemoglobin level, white blood cell count, thrombocyte count, hypertension, total cholesterol, triglycerides, self-reported diabetes, and red blood cell count. During a median follow-up of 15.8 years, 1152 participants experienced a first-ever stroke. A 1% increment in RDW yielded a 13% higher risk of stroke (multivariable HR: 1.13, 95% CI: 1.07-1.20).

Subjects with RDW in the highest quintile compared to the lowest had a 37% higher risk of stroke in multivariable analysis (HR: 1.37, 95% CI: 1.11-1.69). Subjects with RDW above the 95-percentile had 55% higher risk of stroke compared to those in the lowest quintile (HR: 1.55, 95% CI: 1.16-2.06). All risk estimates remained unchanged after exclusion of subjects with anaemia (n=1102). RDW was not associated with increased risk of death within one year or during the entire follow-up after an incident stroke.

RDW is associated with incident stroke in a general population, independent of anaemia and traditional atherosclerotic risk factors.

Keywords: Epidemiological studies, Stroke, Risk Factors

Introduction

Red blood cell distribution width (RDW), an inexpensive and easy accessible measure of the size variability of circulating erythrocytes, is calculated by most automated blood cell counters. RDW gives the coefficient of variation of the red blood cell volume in percentage, and is the numeric equivalent to anisocytosis judged from a peripheral blood smear.

Combined with mean corpuscular volume (MCV), RDW is traditionally used in the differential diagnosis of anaemia. Haematological diseases and conditions that result in premature release of red blood cells into the blood stream also lead to an increase in RDW (1-3).

Recent studies of various selected populations, including patients with heart failure (4-6), patients undergoing percutaneous coronary intervention (7), and male patients referred for coronary angiography (8), have reported an association between RDW and all-cause mortality. Growing evidence is also supporting an association between RDW and cardiovascular morbidity and mortality (9-11). In large cohorts recruited from the general population, high RDW was associated with incident myocardial infarction and fatal coronary events (12, 13). A post hoc analysis of 4111 subjects with previous myocardial infarction from the Cholesterol and Recurrent Event (CARE) study reported a 2.6-fold higher risk of stroke among subjects with RDW in the highest compared to the lowest quartile (14). A case-control study of 224 patients with first-ever ischemic stroke and 224 controls reported a dose-response relation between RDW and risk of stroke, and subjects in the highest RDW quartile had a 5.9-fold higher risk of stroke than subjects in the lowest quartile (15). An association between RDW and stroke was also reported among 133 patients with stable heart failure followed for one year (16). In a registry based study of 41,140 subjects with atrial fibrillation, RDW was found to be directly associated with the risk of stroke, regardless of anaemia status

(17). Furthermore, RDW has been associated with both all-cause and cardiovascular death in subjects with a history of stroke (18, 19).

Cerebrovascular events are the number one cause of permanent disability, and the third most common cause of death in the western world (20-22). Identification of novel biomarkers is an important step to improve risk stratification and thereby potentially prevent disease by targeted interventions. The impact of RDW on the risk of incident stroke in a general population remains unknown. Therefore, we set out to investigate whether elevated RDW was associated with a first-ever stroke and case-mortality in stroke patients in a large cohort recruited from a general population.

Materials and Methods

Study population

Study participants were recruited from the fourth survey of the Tromsø Study, a single-centre prospective, population-based study, conducted in 1994-95. All inhabitants aged 25 years or older were invited. A total of 27158 attended, yielding a participation rate of 77%. The municipality of Tromsø is the largest in northern Norway, and is predominantly inhabited by Caucasians of Norwegian origin. The study population has been described in detail elsewhere (23). We excluded subjects without valid written consent to medical research (n=202), subjects with a history of stroke prior to the survey (n=342), and subjects who were lacking measurements of RDW (n=622). Hence, 25992 participants (12279 men and 13713 women) were enrolled in the present study. The study was approved by the regional committee for research ethics, and informed written consent was obtained from all participants.

Measurements of cardiovascular risk factors

Baseline information on cardiovascular risk factors was obtained through physical examinations, blood samples, and self-administered questionnaires (23).

Blood samples were drawn from an antecubital vein into vacutainer tubes containing EDTA as an anticoagulant (K3-EDTA 40 μ L, 0.37 mol/L per tube). 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%.

Lipid analyses were conducted on serum from non-fasting blood samples. The serum was prepared by centrifugation after a one hour respite at room temperature. Commercial kits were

used to measure triglycerides, total cholesterol, and high density lipoprotein (HDL) cholesterol, as previously described (24). All blood samples were analysed at the Department of Clinical Chemistry, University Hospital of North Norway.

Blood pressure was recorded with an automatic device (Dinamap Vital Signs Monitor, 1846, Critikon Inc., Tampa, FL, USA) by trained personnel. After two minutes rest in a seated position, three recordings were done 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 ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg.

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.

Information on daily smoking and diabetes was obtained from self-administered questionnaires. 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 question on diabetes read as follows: “do you have or have you had diabetes?” (yes/no).

Outcome assessment

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. The Norwegian national 11-digit identification number allowed linkage to national and local diagnosis

registries. First-ever non-fatal and fatal strokes were identified by a search for the International Classification of Diseases (ICD) 9th Revision codes 430-438 and ICD 10th Revision codes I60-I69 in the diagnosis registries at the University Hospital of North Norway (diagnoses from outpatient clinics included), and the National Causes of Death Registry at Statistics Norway. The University Hospital of North Norway is the only hospital serving the community, the nearest hospital being located approx. 250 km away by road (148 by air). The Causes of Death Registry covers all subjects registered as inhabitants of Norway at the time of their death, without regard to whether the death took place in Norway or abroad. This ensured a complete follow-up status for all-cause mortality.

All possible hospitalized and out-of hospital stroke events were validated by an independent endpoint committee. 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. We also performed 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. Person-time of follow-up was calculated from the date of enrolment in 1994-95 to the date when a stroke was first diagnosed, the date a participant died, the date a participant moved from the municipality of Tromsø, or the end of the study period, December 31st 2010, whichever came first. Data was censored from date of death from other causes than stroke (n=3685). For people moving out of Tromsø, the data was censored from date of migration (n=4174).

Case-mortality was defined as overall death or death within one year after incident stroke. For these analyses, person-time was calculated from the date of first-ever stroke to the date of death, migration or study end (Dec 31, 2010) whichever came first.

Statistical analyses

Statistical analyses were carried out using STATA version 13.0 (Stata corporation, College station, TX, USA) and R (version 2.15.1 for Windows). The significance level was set to 0.05. For categorical analyses, the study population was divided into quintiles based on baseline RDW values (Quintile 1: $\leq 12.3\%$, Quintile 2: 12.4-12.6%, Quintile 3: 12.7-12.9%, Quintile 4: 13.0-13.4%, and Quintile 5: $\geq 13.5\%$). Additionally, a cut-off point was fixed at the 95th percentile (RDW values $\geq 14.4\%$). Age-adjusted baseline characteristics across quintiles of RDW were estimated using analysis of variance (ANOVA) for continuous variables and logistic regression for dichotomous variables. Student's t-test and chi square test was used to assess mean differences of baseline characteristics between subjects with and without stroke.

Crude incidence rates (IR) were calculated as the total number of events divided by the total person time, and expressed as number of events per 1000 person-years at risk. Cox proportional hazard regression models were used to estimate age- and sex-adjusted, and multivariable adjusted hazard ratios (HR) with 95% confidence intervals (CI) for stroke per 1% increment in RDW, as well as across quintiles of RDW, or RDW above the 95th percentile. In the percentile-based analyses, the lowest RDW quintile was used as reference group. The multivariable analyses were divided into two models: Model 1 included age, sex, body mass index, daily smoking, haemoglobin, white blood cells, and platelet count. Model 2 included the variables in Model 1, as well as hypertension, total cholesterol, triglycerides, self-reported diabetes, and red blood cell count as these were additional potential

confounders. The number of study participants in the different adjustment models varied slightly due to missing data for some of the co-variables (in total <2% missing).

Additionally, the association between RDW and stroke was visualized by a generalized additive regression plot. In this plot, RDW (log-transformed) was modelled with a 4-degrees of freedom smoothing spline fit in a Cox proportional hazard model using R. Moreover, a plot visualizing the multivariable adjusted cumulative hazard of stroke according to quintiles of RDW was made using the graphing function in Stata.

In subgroup analyses, the association between RDW and risk of ischemic stroke was analysed separately, and HRs were estimated across quintiles of RDW using the same adjustment models as described above.

Lastly, the one year and overall case-mortality rates after incident stroke were estimated according to categories of RDW. Confounder adjustments were assessed using the same models as described above, except for age-adjustments which were carried out using the subjects age at the time of the stroke, rather than their age at baseline. The estimates were also adjusted for the time interval between baseline RDW measurement and the incident stroke. The proportional hazards assumption was tested using Schoenfeld residuals, and no violation was found. Cross-product terms of RDW and sex, or RDW and age, were included in adjustment model 2 to test for statistical interactions. There were no sex or age interactions.

Results

A total of 1152 subjects, of whom 631 were men and 521 women, experienced a first-ever stroke during a total of 340 977 person-years of follow-up. The median follow-up time was 15.8 years and the overall crude incidence rate of stroke was 3.38 (95% CI: 3.19-3.58) per 1000 person-years.

Table 1 shows the age-adjusted distribution of baseline characteristics across quintiles of RDW. Age, the proportion of daily smokers, HDL cholesterol, white blood cell count and platelet count increased with increasing RDW, while haemoglobin levels and the proportion with self-reported diabetes mellitus decreased.

Baseline characteristics of subjects who did (n=1152) and did not (n=24840) develop stroke during follow-up are shown in Table 2. Traditional cardiovascular risk factors such as age, systolic blood pressure, diastolic blood pressure, BMI, total cholesterol, triglycerides, as well as the proportion of males and subjects with diabetes or hypertension were higher in subjects who developed stroke compared to those who did not. The platelet count and the proportion of daily smokers were lower in subjects that experienced a future stroke compared to those who did not.

Hazard ratios and incidence rates across quintiles of RDW are shown in Table 3. The incidence rate increased from 1.5 (95% CI: 1.3-1.8) per 1000 person-years in the lowest quintile, to 6.7 (95% CI: 6.1-7.5) per 1000 person-years in the highest quintile of RDW. High RDW was associated with increased risk of stroke in both age- and sex-adjusted and multivariable analysis. The hazard ratio for stroke was 1.48 (95% CI: 1.21-1.81) when comparing the highest and lowest quintile in age- and sex-adjusted analysis. The association was weakened, but remained significant after adjustments in Model 1 (HR: 1.39, 95% CI: 1.13-1.71), and Model 2 (HR: 1.37, 95% CI: 1.11-1.69). Subjects with RDW above the 95-percentile had 55% higher risk of stroke compared to those in the lowest quintile (Model 2: HR 1.55, 95% CI: 1.16-2.06). Multivariable adjusted cumulative hazards for stroke by increasing quintiles of RDW are shown in Figure 1. There was a dose-response relationship between quintiles of RDW and risk of stroke that progressed over time.

The generalized additive plot showed a clear dose-response relationship between RDW and risk of stroke (Figure 2). When RDW was entered as a continuous variable in the

Cox-model, a 1% increase in RDW was associated with 13% higher risk of stroke after adjustment for age and sex (HR: 1.13, 95% CI: 1.08-1.19). The risk estimate remained essentially similar after multivariable adjustments (Model 1 HR: 1.15, 95% CI: 1.09-1.21) and after additional adjustments for cardiovascular risk factors (Model 2: HR 1.13, 95% CI: 1.07-1.20).

In subgroup analyses, the risk estimates of ischemic stroke according to RDW quintiles were similar to those of total stroke (Table 3). Individuals in the highest RDW quintile had 30% higher risk of ischemic stroke (Model 2: HR 1.30, 95% CI: 1.04-1.64) compared with the reference group.

The risk estimates remained essentially similar after exclusion of subjects with anaemia (n=1102) (Supplementary table 1). The risk of stroke by RDW was somewhat weakened when the analyses were restricted to non-smokers (Model 2 HR for quintile 5 vs. quintile 1: 1.27, 95% CI: 0.98-1.64) (Supplementary table 2). In analyses restricted to smokers, the risk was 60% increased for those with RDW in quintile 5 versus quintile 1 (Model 2: HR 1.60, 95% CI: 1.07-2.49) (Supplementary table 2).

A total of 680 of the subjects that suffered an incident stroke died during follow-up. Hazard ratios of case fatality are shown in Table 4. The crude mortality rate after stroke was 140 (95% CI: 129-150) per 1000 person-years. Subjects in the highest RDW quintile had 96% higher risk of death compared with subjects in the lowest quintile (HR 1.96, 95% CI: 1.47-2.61) in unadjusted analysis (data not shown). The risk estimate was substantially reduced, and no longer statistically significant, when applying adjustment Model 1: HR 1.28, 95% CI 0.95-1.73, and Model 2: HR 1.23, 95% CI 0.91-1.67. Moreover, RDW was not associated with increased risk of death within one year after an incident stroke (Table 4).

Discussion

Our study is, to the best of our knowledge, the first to explore the relationship between RDW and incident stroke, as well as case fatality, in a population-based cohort. RDW, both modelled as a categorical and continuous variable, was associated with increased risk of incident stroke after adjustment for traditional atherosclerotic risk factors. We found no association between RDW and risk of overall mortality or one-year mortality after incident stroke.

Our finding of an association between RDW and risk of incident stroke in a general population is supported by results from observational studies in selected populations. In a study of 41140 subjects with atrial fibrillation, a 33% (HR: 1.33, 95% CI: 1.15-1.53) increased risk of stroke (1692 cases) was shown for subjects in the highest RDW quartile, as compared with the lowest (17). In 133 non-anaemic patients with stable chronic heart failure, patients who developed stroke during follow-up had significantly higher baseline RDW (16). A dose-response relationship between RDW and risk of stroke was found in a case-control study of 224 patients with incident ischemic stroke and 224 controls. Subjects in the highest RDW quartile had an almost 6-fold higher risk of stroke compared with those in the lowest quartile in multivariable analysis (15). In a post hoc analysis of subjects with previous myocardial infarction from the CARE study, the stroke risk was 2.6-fold higher in subjects with RDW in the highest compared with the lowest quartile (14). Similarly, a cross-sectional study of 2497 diabetic patients from the National Health and Nutrition Examination Survey (NHANES) reported that subjects in the highest RDW quartile had a 2.6-fold higher odds of stroke compared with subjects in the lowest quartile (25).

We did not find any association between RDW and all-cause mortality among patients with incident stroke. Several studies have, however, shown an association between RDW and all-cause mortality, as well as cardiovascular mortality, in subjects both with and without previous cardiovascular disease (5, 7, 8, 26, 27). Data from the Malmö Diet and Cancer study

showed that RDW was associated with case fatality in subjects with coronary events (13). The risk of death on the day of the coronary event, as well as within 28 days after the coronary event, increased with increasing quartiles of RDW in multivariable analysis. However, there was no association between RDW and risk of non-fatal coronary events. In a cohort study of 3226 subjects from Taiwan no association between RDW and incident stroke or cardiovascular mortality was found, but a relationship of RDW with all-cause mortality and non-cardiovascular mortality was reported (28). The reasons for the diverging results are not known, but may include differential impact of ethnicity on the associations or differential population characteristics.

In contrast to our findings, two previous studies have reported an association between RDW and mortality in subjects with known cerebrovascular disease. A study by Kim et al. reported that RDW was associated with poor functional outcome and all-cause mortality at three months, and one year after stroke onset (18). Contradictory, in an analysis of 1504 patients with stroke from the Acute Stroke Registry and Analysis of Lausanne (ASTRAL), elevated RDW levels were not associated with poor functional outcome (29). Results from the NHANES study showed that high baseline RDW in subjects with stroke predicted subsequent cardiovascular deaths (HR: 2.38, 95% CI: 1.41-4.01) and all-cause mortality (HR: 2.0, 95% CI: 1.25-3.20) (19). The apparent discrepant findings may rely on the time sequence of RDW measurement and the occurrence of stroke. In both studies reporting a relationship, RDW was measured when the subjects suffered the stroke, whereas we measured RDW on average 7.4 years prior to the cerebrovascular event. Although our analyses were adjusted for time between baseline measurements and the incident stroke, the timing between RDW measurement, stroke and death could have influenced our findings. Other possible explanations for the diverging results may be different population characteristics, size of the populations, and incidences of stroke.

The mechanisms behind the observed relationship between elevated RDW and incident stroke remain uncertain. As RDW is a pure numeric concept calculated from the mean corpuscular volume, it is likely to assume that some underlying condition must be involved

Several studies support the theory that RDW is a marker of inflammation and increases in response to pro-inflammatory cytokines. This aligns well with the notion that inflammation plays an important role in atherogenesis, which is the principal cause of cardiovascular events.(30, 31) In a study of 3845 unselected outpatients, a significant trend was observed for increasing high sensitivity CRP levels and erythrocyte sedimentation rate across increasing quartiles of RDW.(32) The associations remained after exclusion of all subjects above 60 years of age. A correlation between RDW and CRP was also observed in overweight adolescents,(33) in subjects with hypertension,(34) and in patients with Alzheimer's disease.(35) In patients with heart failure, an increase in interleukin-6 and sTNF-receptor was observed with increasing tertiles of RDW.(36)

Various inflammatory cytokines interact with the effect of erythropoietin on the bone marrow, which leads to hypoproliferation,(37) and the production of hepcidin, a regulator of iron homeostasis.(38) A reduced production of erythrocytes, and reduced serum iron levels due to increased hepcidin secretion, may potentially lead to anemia. With the anemia there will be an increase in RDW levels that could explain the link between RDW and inflammatory biomarkers, and the association between RDW and various inflammatory diseases. However, the results from our analyses showed that the risk estimates for stroke remained essentially similar after exclusion of anemic study participants. This gives reason to believe that the observed association in the present study was independent of the link between RDW and anemia of inflammation. Researchers have argued that RDW evaluated alone,

without other inflammatory markers, do not yet give precise enough information for use in clinical practice.(39, 40) For this, further studies are warranted.

RDW might also increase due to iron deficiency independent of anaemia status. Iron deficiency causes increased levels of reactive oxygen species and a reduction in iron-dependent scavenger functions that may promote inflammation (41). Oxidative stress plays an important role in the aetiology of atherosclerosis (42), and can potentially lead to an increase in RDW through an impact on both hematopoietic stem cells and the life span of red blood cells (43). Another way RDW might influence the development of atherosclerosis and subsequent cardiovascular events more directly is through pathologic changes in the erythrocyte membrane, as the size distribution may impact the way the erythrocytes are incorporated into atherosclerotic plaques. Accumulation of erythrocytes, rich in free cholesterol (44), influence both plaque growth and stability (45). We have recently shown that RDW was associated with plaque growth during 7 years of follow-up in a study of 4677 subjects from the general population. The association between RDW and plaque growth was not dependent of traditional atherosclerotic risk factors, including hs-CRP (46). Our findings support the concept that the role of RDW in development and stability of atherosclerotic plaques, either through iron-deficiency or accumulation of erythrocytes, is an important component in the relationship between RDW and cardiovascular events.

Atrial fibrillation is a well-established risk factor for stroke (47, 48). In a study of 27124 subjects from the general population, there was a 33% increased risk of developing atrial fibrillation in the highest RDW quartile compared to the lowest (49). Thus, the observed relation between RDW and stroke could potentially be mediated by atrial fibrillation.

A major strength of our study is the prospective, population-based design with a long follow-up time. The large number of participants with high attendance rate gives good generalizability to the rest of the population. The thorough identification and validation of

stroke events, and only one hospital serving the Tromsø region, decreases the possibility of missed outcomes. All blood samples were analysed in the same laboratory, and well-determined values of baseline characteristics allowed for adjustment for many potential confounders. Some limitations of the study merit consideration. There was only one measurement of RDW throughout the study period, which may have led to underestimation of the associations due to regression dilution bias. Finally, residual confounding cannot be completely ruled out. This may arise from lacking information on inflammatory biomarkers, kidney status, lung status, heart disease and other underlying diseases that might affect baseline RDW.

In conclusion, we found a relationship between increasing RDW and risk of incident stroke in a general population, both when RDW was modelled as a continuous and a categorical variable. The association remained after exclusion of subjects with anaemia, and after adjustments for traditional atherosclerotic risk factors. We found no association between RDW and risk of death after incident stroke. Future studies are warranted to confirm the association between RDW and incident stroke in the general population and to unveil mechanisms linking RDW to risk of stroke.

Author contributions

J. Lappegård – analysed the data and drafted the manuscript

T.S. Ellingsen – interpreted the results and revised the manuscript

T. Skjelbakken – interpreted the results and revised the manuscript

E.B. Mathiesen – collected data, interpreted the results and revised the manuscript

Inger Njølstad – collected data, interpreted the results and revised the manuscript

Tom Wilsgaard – provided statistical support, and revised the manuscript

J. Brox – contributed with data collection, interpreted the results and revised the manuscript

S.K. Brækkan – designed the study, interpreted the results, and revised manuscript

J.B. Hansen - designed the study, interpreted the results, and revised manuscript

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Disclosure

No conflict of interest

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Table legends

Table 1 Age-adjusted baseline characteristics

Age-adjusted baseline characteristics across quintiles of red cell distribution width.

The values are reported as means \pm standard deviation, or percentages with number in brackets.

Table 2 Baseline characteristics stratified according to future development of stroke

Baseline characteristics of study participants stratified according to future development of stroke, with p-value for differences between the groups. The values are reported as means \pm standard deviation, or percentages with number in brackets.

Table 3 Adjusted hazard ratios for stroke

Adjusted hazard ratios for stroke across quintiles of red cell distribution width (RDW)

Incidence rates (IR) and hazard ratios (HRs) with 95% confidence intervals (CI) for total stroke and ischemic stroke according to quintiles of RDW.

Table 4 Adjusted hazard ratios for mortality

Adjusted hazard ratios for overall mortality and mortality within one year after stroke.

Incidence rates (IR) and hazard ratios (HRs) with 95% confidence intervals (CI) for case fatality according to quintiles of RDW

Figure legends

Figure 1

Cumulative hazard of stroke according to quintiles of RDW. The Tromsø Study, 1994-2010. The model is adjusted for age, sex, body mass index, smoking, haemoglobin, white blood cells, thrombocytes, hypertension, cholesterol, triglycerides, self-reported diabetes and red blood cell count.

Figure 2

Dose-response relationship between RDW and risk of stroke obtained by generalized additive regression. The regression model is adjusted for age, sex, body mass index, smoking, haemoglobin, white blood cells, thrombocytes, hypertension, cholesterol, triglycerides, self-reported diabetes and red blood cell count. The solid line shows HRs and the shaded area shows 95% CI. The density plot shows the distribution of RDW and the white vertical lines indicate 2.5th, 25th, 50th, 75th and 97.5th percentiles.

Table 1. Age-adjusted baseline characteristics

	<i>Quintile 1</i>	<i>Quintile 2</i>	<i>Quintile 3</i>	<i>Quintile 4</i>	<i>Quintile 5</i>	<i>95th percentile</i>
	<i>(n=6532)</i>	<i>(n=5070)</i>	<i>(n=4693)</i>	<i>(n=5231)</i>	<i>(n=4466)</i>	<i>(n=1439)</i>
RDW range	10.7-12.3	12.4-12.6	12.7-12.9	13.0-13.4	13.5-30.5	>14.3
Age, years	40.7 ± 12.2	44.2 ± 13.7	46.7 ± 14.1	50.1 ± 15.1	53.6 ± 16.0	53.3 ± 16.6
Sex, % males	46.0	48.5	50.2	49.0	42.5	31.9
Current smoking, %	28.2 (1842)	33.1 (1678)	37.0 (1736)	44.1 (2307)	46.8 (2090)	41.1 (591)
Body mass index, kg/m ²	25.0 ± 3.5	25.2 ± 3.7	25.2 ± 3.7	25.2 ± 3.9	25.2 ± 4.3	25.0 ± 4.4
Systolic blood pressure, mmHg	134 ± 17	135 ± 19	134 ± 20	134 ± 21	134 ± 24	134 ± 24
Diastolic blood pressure, mmHg	78 ± 11	78 ± 12	78 ± 12	78 ± 13	78 ± 14	78 ± 14
Hypertension, %	33.4 (2182)	34.0 (1724)	33.0 (1549)	32.6 (1705)	34.3 (1532)	34.3 (494)
Total cholesterol, mmol/L	5.96 ± 1.22	6.04 ± 1.28	6.08 ± 1.31	6.1 ± 1.32	6.03 ± 1.35	5.84 ± 1.28
HDL cholesterol, mmol/L	1.48 ± 0.39	1.48 ± 0.40	1.49 ± 0.40	1.51 ± 0.41	1.55 ± 0.44	1.59 ± 0.45
Triglycerides, mmol/L	1.6 ± 1.1	1.6 ± 1.1	1.6 ± 1.1	1.5 ± 1.0	1.4 ± 1.0	1.3 ± 0.8
Self-reported diabetes, %	2.2 (144)	1.7 (86)	2.1 (99)	1.4 (73)	1.2 (54)	1.7 (24)
White Blood cells, x10 ⁹ /L	6.86 ± 1.81	6.98 ± 1.92	7.13 ± 2.13	7.26 ± 2.01	7.43 ± 2.23	7.31 ± 2.49
Thrombocytes, x10 ⁹ /L	249 ± 51	248 ± 52	250 ± 54	254 ± 55	264 ± 68	283 ± 81
Red blood cells, x10 ¹² /L	4.63 ± 0.42	4.65 ± 0.41	4.65 ± 0.41	4.64 ± 0.41	4.60 ± 0.43	4.55 ± 0.45
Haemoglobin, g/dL	14.2 ± 1.1	14.2 ± 1.1	14.1 ± 1.1	14.0 ± 1.1	13.5 ± 1.4	12.7 ± 1.6
Mean corpuscular volume, fL	89.4 ± 3.4	89.2 ± 3.5	89.0 ± 3.7	89.0 ± 3.9	86.9 ± 6.2	83.2 ± 8.1

Table 2. Baseline characteristics stratified according to future development of stroke

Stroke	Without	With	P value
N	24840	1152	
Age, years	45.7 ± 14.4	64.0 ± 12.7	<0.001
Sex, % males	46.9	53.5	<0.001
RDW, %	12.9 ± 0.9	13.3 ± 1.1	<0.001
Systolic Blood Pressure, mmHg	133 ± 19	155 ± 25	<0.001
Diastolic Blood Pressure, mmHg	77 ± 12	87 ± 14	<0.001
Hypertension, %	31.4 (7761)	73.5 (936)	<0.001
Body Mass Index, kg/m ²	25.1 ± 3.8	26.5 ± 4.2	<0.001
Total Cholesterol, mmol/L	6.0 ± 1.3	6.7 ± 1.3	<0.001
High density lipoprotein cholesterol, mmol/L	1.5 ± 0.4	1.5 ± 0.4	0.008
Triglycerides, mmol/L	1.5 ± 1.0	1.8 ± 1.1	<0.001
Daily smoking, %	37.0 (9146)	35.5 (452)	0.053
Self-reported diabetes %	1.5 (371)	5.4 (69)	<0.001
Haemoglobin (total), g/dL	14.0 ± 1.2	14.2 ± 1.2	<0.001
Mean corpuscular volume, fL	88.8 ± 4.2	89.2 ± 4.4	<0.001
Red blood cells, x10 ¹² /L	4.6 ± 0.4	4.7 ± 0.4	<0.001
White blood cells, x10 ⁹ /L	7.1 ± 2.0	7.2 ± 1.9	0.2
Thrombocytes, x10 ⁹ /L	253 ± 56	247 ± 58	<0.001

Table 3. Adjusted hazard ratios for stroke

RDW	Person years	No of events	Crude IR (95% CI)	Age/sex adjusted HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Total stroke						
Quintile 1	87930	136	1.5 (1.3-1.8)	Ref	Ref	Ref
Quintile 2	68196	169	2.5 (2.1-2.9)	1.08 (0.86-1.36)	1.06 (0.84-1.33)	1.06 (0.85-1.34)
Quintile 3	62737	198	3.2 (2.7-3.6)	1.10 (0.88-1.37)	1.05 (0.84-1.32)	1.05 (0.84-1.31)
Quintile 4	68405	287	4.2 (3.7-4.7)	1.11 (0.90-1.37)	1.06 (0.86-1.31)	1.07 (0.87-1.33)
Quintile 5	53708	362	6.7 (6.1-7.5)	1.48 (1.21-1.81)	1.39 (1.13-1.71)	1.37 (1.11-1.69)
P (trend)			<0.001	<0.001	0.002	0.003
>95 percentile	14833	99	6.7 (5.5-8.1)	1.65 (1.27-2.15)	1.67 (1.26-2.20)	1.55 (1.16-2.06)
Ischemic stroke						
Quintile 1	87930	123	1.4 (1.2-1.7)	Ref	Ref	Ref
Quintile 2	68196	142	2.1 (1.8-2.5)	1.00 (0.79-1.28)	0.98 (0.77-1.25)	0.99 (0.77-1.26)
Quintile 3	62737	172	2.7 (2.4-3.2)	1.05 (0.83-1.33)	1.01 (0.80-1.27)	1.00 (0.79-1.27)
Quintile 4	68405	251	3.7 (3.2-4.2)	1.07 (0.86-1.34)	1.02 (0.82-1.27)	1.04 (0.83-1.30)
Quintile 5	53708	310	5.8 (5.2-6.4)	1.40 (1.12-1.73)	1.30 (1.04-1.63)	1.30 (1.04-1.64)
P (trend)			<0.001	0.001	0.009	0.010
>95 percentile	14833	89	6.0 (4.9-7.4)	1.64 (1.24-2.16)	1.67 (1.24-2.23)	1.60 (1.18-2.17)

Model 1: Age, sex, BMI, smoking, haemoglobin, white blood cell count, thrombocytes

Model 2: Model 1 + hypertension, cholesterol, triglycerides, self-reported diabetes and red blood cell count

Table 4. Adjusted hazard ratios for mortality

RDW	N	Person years	No of deaths	Crude IR (95% CI)	Age/sex adjusted HR (95% CI)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Overall mortality							
Quintile 1	136	662	58	88 (68-113)	Ref	Ref	Ref
Quintile 2	168	745	79	116 (85-132)	0.96 (0.69-1.35)	0.91 (0.64-1.28)	0.89 (0.63-1.26)
Quintile 3	196	906	108	119 (99-144)	1.05 (0.76-1.44)	1.01 (0.73-1.39)	1.02 (0.73-1.41)
Quintile 4	285	1078	175	163 (141-189)	1.23 (0.91-1.66)	1.16 (0.85-1.57)	1.17 (0.85-1.59)
Quintile 5	357	1499	260	174 (154-196)	1.30 (0.97-1.73)	1.28 (0.95-1.73)	1.32 (0.97-1.80)
>95 percentile	98	403	78	194 (155-242)	1.31 (0.93-1.84)	1.33 (0.93-1.90)	1.34 (0.92-1.94)
1 year mortality							
Quintile 1	136	116	20	172 (111-266)	Ref	Ref	Ref
Quintile 2	168	142	27	190 (130-277)	0.86 (0.48-1.54)	0.85 (0.47-1.52)	0.87 (0.48-1.57)
Quintile 3	196	163	34	208 (149-291)	0.95 (0.54-1.64)	1.00 (0.57-1.75)	0.98 (0.56-1.72)
Quintile 4	285	228	61	268 (208-344)	1.06 (0.64-1.76)	1.13 (0.67-1.89)	1.08 (0.64-1.82)
Quintile 5	357	295	80	271 (218-337)	1.05 (0.64-1.73)	1.13 (0.67-1.88)	0.96 (0.56-1.62)
>95 percentile	98	77	27	350 (240-511)	1.25 (0.70-2.25)	1.31 (0.71-2.41)	1.01 (0.53-1.93)

Model 1: Age at time of stroke, sex, BMI, smoking, haemoglobin, white blood cell count, thrombocytes

Model 2: Model 1 + hypertension, cholesterol, triglycerides, self-reported diabetes, red blood cell count and time from baseline measurement to incident stroke

Figure 1

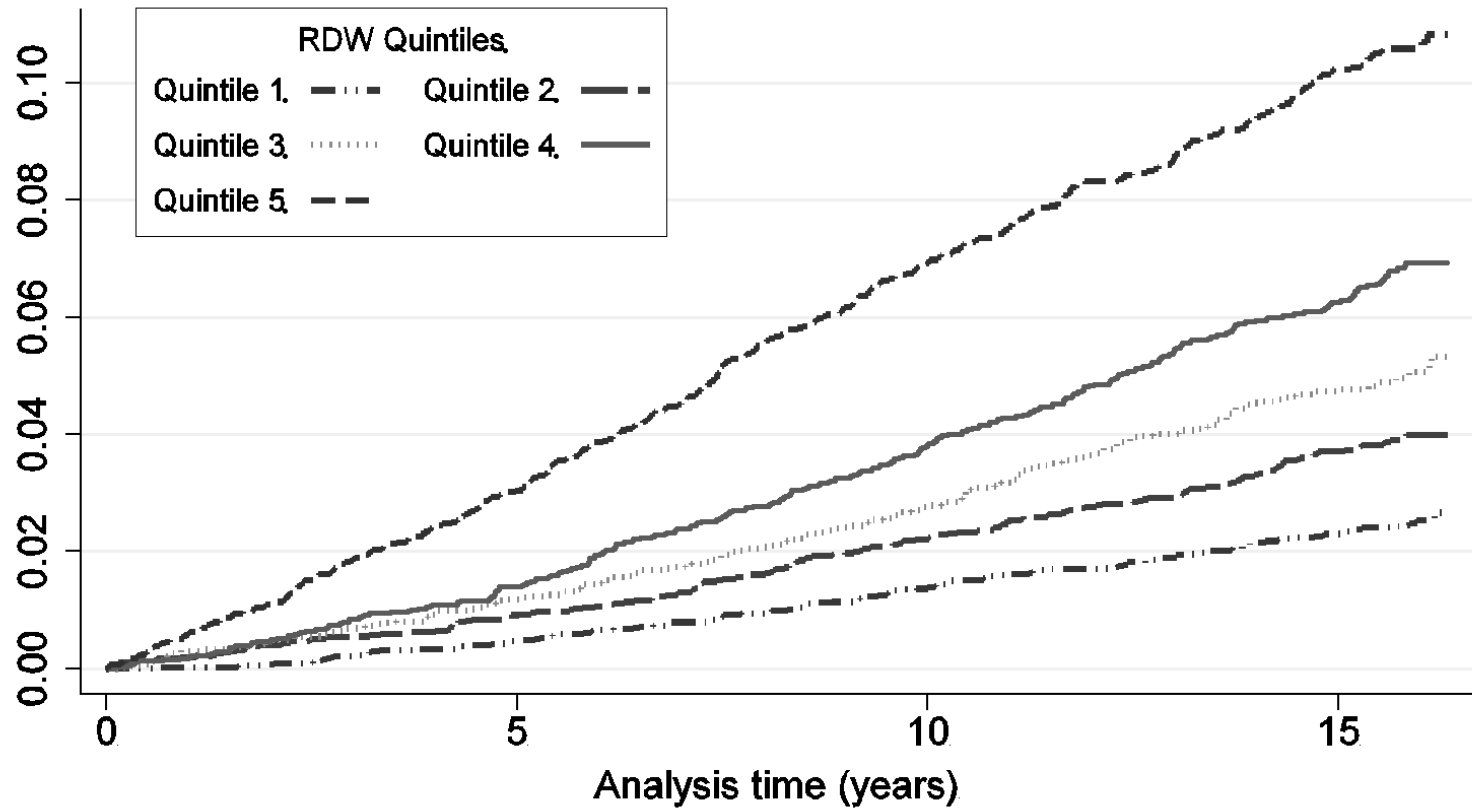


Figure 2

RDW AND STROKE

