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Red Cell Distribution Width in Patients with Diabetes and Myocardial Infarction: an analysis from the EXAMINE trial

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Abstract

**Background:** Red blood cell distribution width (RDW) is a measure of size variability in the red blood cell population (anisocytosis). Increased RDW may arise from any condition that affects erythropoiesis or the survival of erythrocytes. RDW has been associated with poor prognosis in patients with type 2 diabetes (T2D). Whether RDW is a risk marker for adverse cardiovascular outcomes or also a marker of non-cardiovascular health concerns is of clinical importance.

**Aims:** To determine the clinical correlates of increased RDW, its potential mechanistic association with multiple circulating biomarkers, and its prognostic value, in patients with (T2D) who had a recent acute coronary syndrome.

**Methods:** We used time-updated Cox models applied to patients enrolled in the EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care) trial.

**Results:** A total of 5380 patients were included, the median age was 61 years and 32% were women. Patients with higher RDW were older, more frequently women, with longer duration of diabetes duration, and increased comorbidities. An RDW >16.1% (both baseline and time-updated) was independently associated with the study primary composite outcome of nonfatal myocardial infarction, nonfatal stroke or cardiovascular death (time-updated adjusted HR =1.36, 95%CI =1.16-1.61, p <0.001), all-cause death (time-updated adjusted HR =2.01, 95%CI =1.60-2.53, p <0.001), as well as mortality from non-CV causes (time-updated adjusted HR =2.67, 95%CI =1.72-4.15, p <0.001). RDW had a weak-to-moderate correlation with hemoglobin and circulating markers that reflected inflammation, apoptosis, fibrosis and congestion. Alogliptin did not alter RDW values.

**Conclusions:** RDW is a marker of disease severity associated with a multitude of poor outcomes, including both cardiovascular and non-cardiovascular death. RDW correlated
modestly with inflammatory, pro-apoptotic, pro-fibrotic, and congestion markers, and its levels were not affected by alogliptin during the course of the trial.

*Key-words:* type 2 diabetes; red cell distribution width; alogliptin; outcomes.
**Introduction**

Red blood cell distribution width (RDW) is a measure of size variability in the red blood cell population (anisocytosis). The RDW value is obtained by dividing the standard deviation of erythrocyte volume by the mean corpuscular volume\(^1\).

Increased RDW reflects higher variability in the size of erythrocytes that may arise from conditions that affect erythropoiesis or the survival of erythrocytes\(^2\)\(^-\)\(^5\). As such conditions are linked with iron and folate deficiencies and systemic inflammation, RDW has been associated with poor prognosis in many clinical scenarios and populations, including patients with type 2 diabetes\(^4\)\(^-\)\(^14\) and metabolic syndromes\(^15\), \(^16\). Such findings are of potential interest because RDW is widely available as part of the routine complete blood count.

Based on the aforementioned associations, a better understanding of an increased RDW is needed in the clinical cardiovascular setting. Using a large cohort of patients with type 2 diabetes (T2D) who had a recent acute coronary syndrome with RDW values available at each study visit over a median period of 18 months, we sought to determine the clinical correlates of increased RDW, its association with multiple circulating biomarkers, and its prognostic value.

**Methods**

**Study design**

Details of the EXAMINE study design have been previously published\(^17\), \(^18\). A total of 5380 patients with T2D who had a recent acute coronary syndrome (ACS) were randomly assigned to either alogliptin or placebo. In the overall population, alogliptin significantly
reduced glycated hemoglobin (HBA1c) without increasing the rates of major adverse cardiovascular events.

The steering committee, consisting of academic members and three nonvoting representatives of the sponsor (Takeda Development Center Americas), designed and oversaw the conduct of the trial. An independent data and safety monitoring committee monitored the trial and had access to the unblinded data.

**Study patients**

Patients were eligible for enrolment if they had received a diagnosis of T2D, were receiving antidiabetic therapy (other than a DPP-4 inhibitor or GLP-1 analogue), and had had an acute coronary syndrome within 15 to 90 days before randomization. Acute coronary syndromes included acute myocardial infarction (MI) and unstable angina requiring hospitalization. Major exclusion criteria were a diagnosis of type 1 diabetes, unstable cardiac disorders (e.g., NYHA class IV heart failure, refractory angina, uncontrolled arrhythmias, critical valvular heart disease, or severe uncontrolled hypertension), and dialysis within 14 days before screening.

Institutional Review Board approval was obtained and all patients provided informed consent to participate in the trial. The EXAMINE trial is registered in ClinicalTrials.gov with the number NCT00968708. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**End points**

The primary endpoint was a composite of death from cardiovascular (CV) causes, nonfatal MI, or nonfatal stroke. In the present analysis we also studied heart failure endpoints, including a composite of CV death or HF hospitalization, and each individual component of the primary outcome, mortality from non-CV causes (non-CV death), and all-cause mortality.
(ACM). An independent central adjudication committee adjudicated all endpoints. The median follow-up time was 1.6 (1.0-2.2) years.

**Clinical and biomarker measurements**

Medical history and a physical examination were all recorded at baseline. A large protein biomarker panel with 92 biomarkers from a wide range of pathophysiological domains was measured (Olink® CVDII panel) at baseline (randomization). We selected this panel because it contains known human cardiovascular and inflammatory markers as well as some exploratory human proteins which may have potential as new markers of cardiovascular disease. These proteins are involved in multiple disease processes such as immune/inflammatory response, angiogenesis, coagulation and cell adhesion, for example. In short, the biomarkers were measured using a high-throughput technique using the Olink Proseek® Multiplex CVD II 96x96 kit, which measures 92 manually-selected cardiovascular-related proteins simultaneously in 1μl plasma samples. The kit uses a proximity extension assay technology, where 92 oligonucleotide-labeled antibody probe pairs are allowed to bind to their respective target present in the sample. The platform provides Log2 normalized protein expression (NPX) data wherein a high protein value corresponds to a high protein concentration, but not an absolute quantification. For further details please go to https://www.olink.com/. In addition to the above referenced Olink® biomarkers, high-sensitivity troponin I (hsTnI; troponin) was measured on the ARCHITECT i2000SR analyzer (Abbott Laboratories®). RDW was available at baseline (randomization) and at all subsequent study visits (i.e., 1, 3, 6, 9, and 12 months, and every 4 months thereafter). If a RDW value was missing the last available value was carried forward.

**Statistical analyses**
First, we tested whether RDW had a linear relationship with the study primary outcome by categorizing the variable in quintiles and by inspection of restricted cubic splines. This preliminary assessment demonstrated that RDW had a non-linear association with all-cause death whereby the associated mortality risk was only significantly increased above 16.1%. The value above 16.1% was higher than the typical reference range for RDW in most laboratories (11.0 to 15.0%\textsuperscript{19}). Hence, using an RDW above 16.1% allows a clear differentiation between patients with normal and abnormal RDW values. The characteristics of the patients with RDW >16.1% vs. ≤16.1% were then compared using descriptive statistics for continuous and categorical variables. The prognostic associations of RDW (categorical and spline-transformed continuous) were studied using survival models, both with the baseline and time-updated RDW values. The models were both crude and adjusted on age, sex, smoking, diabetes duration, previous MI, previous HF hospitalization, previous stroke, atrial fibrillation (AFib), hypertension, systolic blood pressure, statin therapy, estimated glomerular filtration rate (eGFR based on the CKD-EPI formula\textsuperscript{20}), anemia status, and study treatment (alogliptin or placebo) allocation. The selection of the variables to include in the clinical model was based on their clinical relevance and previous publications from the EXAMINE dataset\textsuperscript{21-26}. We have used “competing risks” models (as described by Fine & Gray\textsuperscript{27}) in cases where the sub-distribution hazard might be affected by a preceding fatal event, as it might have occurred for the outcomes of myocardial infarction, stroke or heart failure hospitalization. The correlation of baseline RDW with circulating Olink® proteins and hemoglobin was performed using Spearman Rho statistics with correlation Rho values below 0.20 considered “very weak correlation”, correlation Rho values between 0.20 and 0.30 considered “weak correlation”, and correlation Rho values between 0.31 and 0.50 considered “modest correlation”; no Rho correlation values above 0.50 were observed.
Mixed-effects models were used to study the effect of alogliptin on RDW values over time. All statistical analyses were performed with Stata® (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC).

Results

Patient characteristics

There were 5380 patients randomized in the EXAMINE trial\textsuperscript{17}. Characteristics of the total study population have been reported previously\textsuperscript{17}. Patients with RDW >16.1% were older, more often female, had longer durations of type 2 diabetes, higher rates on insulin, with HF, stroke, atrial fibrillation and peripheral artery disease. In addition, those with RDW >16.1 had lower hemoglobin levels and eGFR, and higher troponin I and brain natriuretic peptide (BNP) levels. \textit{Table 1}.

Outcome associations

An RDW >16.1% (both baseline and time-updated, categorical and continuous) was independently associated with the study primary composite outcome of nonfatal MI, nonfatal stroke or CV death (time-updated adjusted HR =1.36, 95% CI =1.16-1.61, p <0.001), all-cause death (time-updated adjusted HR =2.01, 95% CI =1.60-2.53, p <0.001), hospitalizations and mortality from CV causes, and also mortality from non-CV causes (time-updated adjusted HR =2.67, 95% CI =1.72-4.15, p <0.001). \textit{Table 2 & Figure 1}. RDW (above or below 16.1%) did not modify the neutral effect of alogliptin on the study primary outcome (p for interaction =0.36).

Biomarker correlations

Baseline RDW was modestly and negatively correlated with baseline hemoglobin (Rho =-0.31), and weakly and positively correlated with baseline TRAIL receptor 2 or death receptor.
5 (TRAILR2 Rho =0.26), fibroblast growth factor 23 (FGF23 Rho =0.24), tumor necrosis factor receptor soluble fraction 11A (TNFRS11A Rho =0.23), tumor necrosis factor receptor soluble fraction 10A (TNFRS10A Rho =0.22), galectin 9 (Gal9 Rho =0.22), spondin-2 (SPON2 Rho =0.22), brain natriuretic peptide (BNP Rho =0.22), cluster of differentiation 4 (CD4 Rho =0.22), and adrenomedullin (ADM Rho =0.20). Table 3. All the other biomarkers, including C-reactive protein, showed very weak correlations with RDW (Rho <0.2). Supplemental Table 1.

Effect of alogliptin on RDW over time

Alogliptin did not alter the RDW values throughout the follow-up period of the EXAMINE trial: global effect =-0.05 (-0.13 to 0.04), p =0.32. Figure 2.

Discussion

Our study shows that in patients with T2D who had a recent ACS, a high RDW (above 16.1%) is a marker of disease severity associated with a multitude of poor outcomes, including both cardiovascular and non-cardiovascular death. RDW was negatively correlated with hemoglobin, and positively correlated with circulating inflammatory markers, BNP and ADM, but the correlations were modest.

Compared with matched healthy controls, patients with type 2 diabetes had higher RDW values, but RDW was not significantly correlated with fasting plasma glucose, HBA1c, or the duration of diabetes, suggesting that, in patients with diabetes, RDW may reflect underlying disease processes that are independent from glucose control. In another large sample of patients with diabetes (n =3,061), patients with higher RDW (>13.7%) were older and with more co-morbid conditions, including cardiovascular and chronic kidney disease. After adjustment for important confounders, a higher RDW remained independently
associated with all-cause and cardiovascular mortality\(^6\). However, Al-Kindi et al\(^6\) did not test whether RDW was also associated with mortality from non-cardiovascular causes. In addition, the patient-population included in the study of Al-Kindi and coworkers was younger and with fewer co-morbid conditions compared to the patients included in the EXAMINE trial. Particularly, the proportion of patients who had an MI ranged from 6 to 20%, whereas in EXAMINE patients had to have an acute coronary syndrome for inclusion in the study. These differences in the severity of the population may explain why the RDW risk cut-point was higher in EXAMINE, and further supports the role of RDW as a non-specific marker of disease severity, rather than a population or outcome-specific marker.

It has been suggested that RDW is a marker that may reflect an underlying inflammatory state\(^9,28,29\). In an unselected cohort of adult outpatients, c-reactive protein and erythrocyte sedimentation rate were higher among patients with higher RDW values\(^28\). In EXAMINE, RDW was negatively correlated with hemoglobin (i.e., the higher the RDW the lower the hemoglobin), which, despite being the top correlation was only moderate (Spearman Rho =0.3). Following, the correlations with TRAILR2, FGF23, TNF receptor family, Gal-9, CD4, BNP and ADM were even weaker (Spearman Rho between 0.2 and 0.3), but suggest that RDW may, at least partly, be correlated with inflammatory, pro-apoptotic, pro-fibrotic and congestion markers. For example, the TRAILR2 protein (otherwise known as death receptor 5) is a member of the TNF receptor superfamily encoded by the \textit{TNFSF10} gene and is a receptor that preferentially induces apoptosis after binding of its ligand TRAIL\(^30,31\). Increased levels of TRAILR2 have been associated with fatal events in patients with MI, possibly due to intensified apoptotic activity driven by TRAIL/TRAILR2\(^32,33\). It was recently found that TRAILR2 was also strongly associated with new-onset HF via mechanisms linked to inflammation and apoptosis\(^34\). FGF23 is released by the osteocytes
and is essential for the regulation of the metabolism of phosphate, calcium and vitamin D. Importantly, FGF23 promotes myocardial fibrosis and has been associated with coronary heart disease and HF\textsuperscript{34-36}. Gal-9 is produced by the extracellular matrix and may be increased in patients with ischemic cardiovascular disease\textsuperscript{37}. Gal-9 has also been previously identified as strongly associated with new-onset HF\textsuperscript{34}, via mechanisms linked to fibrosis and extra-cellular matrix remodeling. CD4 (T-cell surface glycoprotein CD4) functions to initiate or increase inflammatory response by activating T-cells\textsuperscript{38}. ADM is a vasodilator peptide predominantly produced by the vascular endothelium and smooth muscle that increases in response to hemodynamic stress\textsuperscript{39}. More recently, it has been suggested that ADM may be well correlated with extra-vascular congestion\textsuperscript{40}. BNP is the sole strongest prognosticator in HF and also in patients who had an MI, including those enrolled in EXAMINE\textsuperscript{26}.

Alogliptin (vs. placebo) did not change the RDW values over time, suggesting that this DPP-4 inhibitor does not affect the pathways related to RDW, including erythrocyte metabolism. These findings may help in part to explain the lack of morbidity and mortality benefit of DPP-4 inhibitors in patients with diabetes, despite their glucose-lowering effects\textsuperscript{17}. In this regard, it would be of interest to determine whether sodium-glucose cotransporter-2 inhibitors (SGLT2i) might decrease RDW levels over time; this hypothesis is plausible since SGLT2i increase hemoglobin/hematocrit, possibly through hypoxia-induced factors\textsuperscript{41}.

Our findings suggest that in patients with T2D and a recent ACS, RDW is a non-specific marker of disease severity; therefore, RDW can be easily implemented in clinical practice (by looking at the complete blood count) to help in a rapid assessment of the patients’ prognosis, particularly regarding the risk of death from any cause.

Limitations
This is a post-hoc analysis of a prospective randomized trial, therefore all limitations inherent to such analysis are applied herein, including the inability to infer causality. Nonetheless, the extensive replication of similar findings in other cohorts strengthen our results. To the best of our knowledge, the association of RDW with non-cardiovascular mortality is novel and should be further replicated.

Conclusions

In patients with T2D who had an ACS, a high RDW above 16.1% is a non-specific marker of disease severity associated with a multitude of poor outcomes, including both cardiovascular and non-cardiovascular death. RDW is related to inflammatory, pro-apoptotic, pro-fibrotic, and vascular congestion markers and its levels were not affected by alogliptin during the course of the EXAMINE trial.

Availability of Data and Materials

The data and materials may be available upon reasonable request.

Funding

The EXAMINE study was funded by Takeda but the present analysis did not receive funding.

Disclosures

G.L.B., has received personal fees from Takeda Development Center, is a consultant for Merck, Relypsa, and is on the steering committee for international renal/CV outcomes trials for Janssen, Bayer, Vascular Dynamics. W.B.W has received research support from the
National Institute of Aging (NIH) and personal fees from Takeda Development Center (Deerfield, IL, USA) during the conduct of the EXAMINE trial until 2016 (Steering Committee Chair). F.Z has received fees for serving on the board of Boston Scientific; consulting fees from Novartis, Takeda, AstraZeneca, Boehringer Ingelheim, GE Healthcare, Relypsa, Servier, Boston Scientific, Bayer, Johnson & Johnson, and Resmed; and speaking fees from Pfizer and AstraZeneca. All the other authors reported not having potential conflicts of interest relevant to this article.

Acknowledgments

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JPF wrote the manuscript, performed the statistical analysis and made critical revisions; CM contributed to discussion and reviewed/edited the manuscript including the revision of the statistical methods and analysis; GB reviewed/edited the manuscript; PR reviewed/edited the manuscript; WBW reviewed/edited the manuscript; FZ contributed to discussion and reviewed/edited the manuscript.

Dr. João Pedro Ferreira is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
References


Table 1. Patients’ characteristics by baseline red cell distribution width categories

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>≤16.1%</th>
<th>&gt;16.1%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.</td>
<td>4098</td>
<td>1282</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>60.3 ± 9.8</td>
<td>62.6 ± 10.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age &gt;65yr</td>
<td>1362 (33.2%)</td>
<td>545 (42.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>2871 (70.1%)</td>
<td>780 (60.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes duration, years</td>
<td>6.8 (2.6, 13.1)</td>
<td>8.4 (3.3, 15.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HBA1c, %</td>
<td>8.1 ± 1.1</td>
<td>7.9 ± 1.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin</td>
<td>1186 (28.9%)</td>
<td>419 (32.7%)</td>
<td>0.011</td>
</tr>
<tr>
<td>Metformin</td>
<td>2773 (67.7%)</td>
<td>789 (61.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>1934 (47.2%)</td>
<td>569 (44.4%)</td>
<td>0.078</td>
</tr>
<tr>
<td>Thiazolidinedione</td>
<td>99 (2.4%)</td>
<td>32 (2.5%)</td>
<td>0.87</td>
</tr>
<tr>
<td>BMI, Kg/m²</td>
<td>29.5 ± 5.5</td>
<td>29.3 ± 5.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>White</td>
<td>3024 (73.8%)</td>
<td>885 (69.0%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>812 (19.8%)</td>
<td>277 (21.6%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>144 (3.5%)</td>
<td>72 (5.6%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>118 (2.9%)</td>
<td>48 (3.7%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>591 (14.4%)</td>
<td>143 (11.2%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3394 (82.8%)</td>
<td>1075 (83.9%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Prior myocardal infarction</td>
<td>3602 (87.9%)</td>
<td>1132 (88.3%)</td>
<td>0.70</td>
</tr>
<tr>
<td>HF history</td>
<td>1088 (26.5%)</td>
<td>445 (34.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke</td>
<td>256 (6.2%)</td>
<td>132 (10.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAD</td>
<td>363 (8.9%)</td>
<td>151 (11.8%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>241 (5.9%)</td>
<td>135 (10.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Index ACS event type</td>
<td></td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>3177 (77.5%)</td>
<td>989 (77.1%)</td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td>921 (22.5%)</td>
<td>293 (22.9%)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>13.7 ± 1.5</td>
<td>12.7 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anemia</td>
<td>826 (20.2%)</td>
<td>542 (42.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m²</td>
<td>72.6 ± 20.8</td>
<td>65.6 ± 22.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR &lt;60 ml/min/1.73 m²</td>
<td>1054 (25.7%)</td>
<td>511 (39.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsTnI, ng/L</td>
<td>8.2 (4.2, 17.8)</td>
<td>12.1 (5.9, 26.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BNP, ng/L</td>
<td>77.0 (35.8, 164.6)</td>
<td>132.1 (58.9, 278.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>71.0 ± 10.5</td>
<td>72.5 ± 11.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>129.0 ± 16.2</td>
<td>129.0 ± 17.8</td>
<td>0.93</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>76.7 ± 9.5</td>
<td>75.5 ± 10.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>4004 (97.7%)</td>
<td>1228 (95.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>3374 (82.3%)</td>
<td>1037 (80.9%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Statin</td>
<td>3720 (90.8%)</td>
<td>1146 (89.4%)</td>
<td>0.14</td>
</tr>
<tr>
<td>CCB</td>
<td>914 (22.3%)</td>
<td>283 (22.1%)</td>
<td>0.86</td>
</tr>
<tr>
<td>Diuretic</td>
<td>1431 (34.9%)</td>
<td>583 (45.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACEi or ARB</td>
<td>3396 (82.9%)</td>
<td>1015 (79.2%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Alogliptin allocation</td>
<td>2073 (50.6%)</td>
<td>628 (49.0%)</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Legend: RDW, red cell distribution width; BMI, body mass index; HF, heart failure; PAD, peripheral artery disease; eGFR, estimated glomerular filtration rate; hsTnI, high-sensitivity troponin I; BNP, brain natriuretic peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure; CCB, calcium-channel blocker; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.
Table 2. Spearman correlation of RDW with circulating biomarkers at baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rho</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>-0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TRAILR2</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FGF23</td>
<td>0.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNFRSF11A</td>
<td>0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNFRSF10A</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GAL9</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SPON2</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BNP</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADM</td>
<td>0.20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: only weak to moderate correlations are presented (Rho ≥0.2).

Legend: TRAILR2, TRAIL receptor 2 or death receptor 5; FGF23, fibroblast growth factor 23; TNFRS11A, tumor necrosis factor receptor soluble fraction 11A; TNFRS10A, tumor necrosis factor receptor soluble fraction 10A; Gal9, galectin 9; SPON2, spondin-2; BNP, brain natriuretic peptide; CD4, cluster of differentiation 4; ADM, adrenomedullin.
Table 3. Outcome associations of baseline and time-updated RDW categories (>16.1% vs. ≤16.1%)

<table>
<thead>
<tr>
<th>End-point</th>
<th>RDW</th>
<th>Baseline RDW</th>
<th>Time-updated RDW</th>
<th>P-value</th>
<th>Time-updated RDW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤16.1%</td>
<td>&gt;16.1%</td>
<td>Crude model</td>
<td>Adj. model</td>
<td>HR (95%CI)</td>
</tr>
<tr>
<td>Primary end-point</td>
<td>n =4098</td>
<td>n =1281</td>
<td>1.68 (1.43-1.99)</td>
<td>1.24 (1.04-1.49)</td>
<td>0.015</td>
</tr>
<tr>
<td>All-cause death</td>
<td>194 (4.7%)</td>
<td>132 (10.3%)</td>
<td>2.18 (1.75-2.72)</td>
<td>1.48 (1.17-1.88)</td>
<td>0.001</td>
</tr>
<tr>
<td>CV death</td>
<td>145 (3.5%)</td>
<td>97 (7.6%)</td>
<td>2.15 (1.66-2.77)</td>
<td>1.43 (1.09-1.88)</td>
<td>0.01</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>253 (6.2%)</td>
<td>107 (8.3%)</td>
<td>1.38 (1.10-1.73)</td>
<td>1.05 (0.83-1.34)</td>
<td>0.68</td>
</tr>
<tr>
<td>Stroke</td>
<td>40 (1.0%)</td>
<td>21 (1.6%)</td>
<td>1.70 (1.00-2.89)</td>
<td>1.51 (0.86-2.65)</td>
<td>0.15</td>
</tr>
<tr>
<td>HF hosp.</td>
<td>108 (2.6%)</td>
<td>87 (6.8%)</td>
<td>2.64 (1.99-3.51)</td>
<td>1.55 (1.15-2.10)</td>
<td>0.004</td>
</tr>
<tr>
<td>CV death or HF hosp.</td>
<td>236 (5.8%)</td>
<td>166 (12.9%)</td>
<td>2.31 (1.89-2.81)</td>
<td>1.49 (1.21-1.84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CV hosp.</td>
<td>561 (13.7%)</td>
<td>262 (20.4%)</td>
<td>1.54 (1.33-1.79)</td>
<td>1.23 (1.06-1.44)</td>
<td>0.008</td>
</tr>
<tr>
<td>CV death or CV hosp.</td>
<td>624 (15.2%)</td>
<td>300 (23.4%)</td>
<td>1.59 (1.38-1.82)</td>
<td>1.24 (1.07-1.43)</td>
<td>0.004</td>
</tr>
<tr>
<td>Non-CV death</td>
<td>49 (1.2%)</td>
<td>35 (2.7%)</td>
<td>2.29 (1.48-3.53)</td>
<td>1.70 (1.08-2.68)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Legend: CV, cardiovascular; HF, heart failure; Hosp., hospitalization; Adj., adjusted on age, sex, smoking, diabetes duration, previous MI, previous HF hospitalization, previous stroke, atrial fibrillation, hypertension, systolic blood pressure, statin therapy, estimated glomerular filtration rate, anemia status, and study treatment (alogliptin or placebo) allocation.
Figure 1. Association of baseline RDW with the study outcomes

Legend: The primary end-point is a composite of cardiovascular death, myocardial infarction or stroke. Models adjusted on age, sex, smoking, diabetes duration, previous MI, previous HF hospitalization, previous stroke, atrial fibrillation, hypertension, systolic blood pressure, statin therapy, estimated glomerular filtration rate, anemia status, and study treatment (alogliptin or placebo) allocation.
Figure 2. Effect of alogliptin (vs. placebo) on the RDW values throughout the follow-up.

Legend: RDW, red cell distribution width.
Caption: RDW did not change RDW over time: global effect = -0.05% (-0.13 to 0.04), p = 0.32.