



HAL
open science

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

Joao Pedro Ferreira, Zohra Lamiral, George Bakris, Cyrus Mehta, William B
White, Faiez Zannad

► **To cite this version:**

Joao Pedro Ferreira, Zohra Lamiral, George Bakris, Cyrus Mehta, William B White, et al.. Red Cell Distribution Width in Patients with Diabetes and Myocardial Infarction: an analysis from the EXAMINE trial. *Diabetes, Obesity and Metabolism*, 2021, 10.1111/dom.14371 . hal-03169870

HAL Id: hal-03169870

<https://hal.univ-lorraine.fr/hal-03169870>

Submitted on 15 Mar 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

João Pedro Ferreira, MD¹; Zohra Lamiral, Msc¹; George Bakris, MD²; Cyrus Mehta, PhD³;
William B. White, MD⁴; Faiez Zannad, MD¹

¹ Université de Lorraine, Centre d'Investigations Cliniques Plurithématique Inserm 1433, Nancy, France, CHRU de Nancy, Inserm U1116, Nancy, France, FCRIN INI-CRCT, Nancy, France.

² Department of Medicine, University of Chicago Medicine, Chicago, IL.

³ Cytel, Incorporated, Cambridge Massachusetts, USA.

⁴ Calhoun Cardiology Center, University of Connecticut School of Medicine, Farmington, Connecticut, USA.

Correspondence to:

Professor Faiez Zannad or Dr João Pedro Ferreira

Centre d'Investigation Clinique 1433 module Plurithématique, CHRU Nancy - Hopitaux de Brabois

Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu

4 rue du Morvan, 54500 Vandoeuvre les Nancy

Tel : +33 (0) 3 83 15 73 15

Fax : +33 (0) 3 83 15 73 24

Mail: f.zannad@chru-nancy.fr or j.ferreira@chru-nancy.fr

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 volume¹.

Increased RDW reflects higher variability in the size of erythrocytes that may arise from conditions that affect erythropoiesis or the survival of erythrocytes²⁻⁵. 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 diabetes⁴⁻¹⁴ 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%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 formula²⁰), 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 dataset²¹⁻²⁶. We have used “competing risks” models (as described by Fine & Gray²⁷) 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 trial¹⁷. Characteristics of the total study population have been reported previously¹⁷. 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. **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). **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 control¹⁵. 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 disease⁶. After adjustment for important confounders, a higher RDW remained independently

associated with all-cause and cardiovascular mortality⁶. However, Al-Kindi et al⁶ 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 values²⁸. 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 *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 apoptosis³⁴. 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 HF³⁴⁻³⁶. Gal-9 is produced by the extracellular matrix and may be increased in patients with ischemic cardiovascular disease³⁷. Gal-9 has also been previously identified as strongly associated with new-onset HF³⁴, 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-cells³⁸. ADM is a vasodilator peptide predominantly produced by the vascular endothelium and smooth muscle that increases in response to hemodynamic stress³⁹. More recently, it has been suggested that ADM may be well correlated with extra-vascular congestion⁴⁰. BNP is the sole strongest prognosticator in HF and also in patients who had an MI, including those enrolled in EXAMINE²⁶.

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 effects¹⁷. 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 factors⁴¹.

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

EXAMINE is a clinical trial sponsored by Takeda Global Research and Development Center, Inc., Deerfield, IL. The authors are solely responsible for the design of the study, all study analyses, and the drafting and editing of the manuscript and its final contents.

Clinical trials identifier: [Clinicaltrials.gov-NCT00968708](https://clinicaltrials.gov/ct2/show/study/NCT00968708).

JPF, PR, FZ are supported by a public grant overseen by the French National Research Agency (ANR) as part of the second “Investissements d’Avenir” programme (ANR-15-RHU-0004). All the other authors have nothing to disclose with regards to this manuscript.

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

1. Morris, M.; Davey, F.; Henry, J., Basic examination of blood. In Clinical diagnosis and management by laboratory methods. . 20th edition. Philadelphia: WB Saunders Company 2001.
2. Forhecz, Z.; Gombos, T.; Borgulya, G.; Pozsonyi, Z.; Prohaszka, Z.; Janoskuti, L., Red cell distribution width in heart failure: prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state. *Am Heart J* **2009**, *158* (4), 659-66.
3. Romero Artaza, J.; Carbia, C. D.; Ceballo, M. F.; Diaz, N. B., [Red cell distribution width (RDW): its use in the characterization of microcytic and hypochromic anemias]. *Medicina (B Aires)* **1999**, *59* (1), 17-22.
4. Pascual-Figal, D. A.; Bonaque, J. C.; Redondo, B.; Caro, C.; Manzano-Fernandez, S.; Sanchez-Mas, J.; Garrido, I. P.; Valdes, M., Red blood cell distribution width predicts long-term outcome regardless of anaemia status in acute heart failure patients. In *Eur J Heart Fail*, Netherlands, 2009; Vol. 11, pp 840-6.
5. Ferreira, J. P.; Girerd, N.; Arrigo, M.; Medeiros, P. B.; Ricardo, M. B.; Almeida, T.; Rola, A.; Tolppanen, H.; Laribi, S.; Gayat, E.; Mebazaa, A.; Mueller, C.; Zannad, F.; Rossignol, P.; Aragão, I., Enlarging Red Blood Cell Distribution Width During Hospitalization Identifies a Very High-Risk Subset of Acutely Decompensated Heart Failure Patients and Adds Valuable Prognostic Information on Top of Hemoconcentration. *Medicine (Baltimore)* **2016**, *95* (14), e3307.
6. Al-Kindi, S. G.; Refaat, M.; Jayyousi, A.; Asaad, N.; Al Suwaidi, J.; Abi Khalil, C., Red Cell Distribution Width Is Associated with All-Cause and Cardiovascular Mortality in Patients with Diabetes. *Biomed Res Int* **2017**, *2017*, 5843702.
7. Saliba, W.; Barnett-Griness, O.; Elias, M.; Rennert, G., The association between red cell distribution width and stroke in patients with atrial fibrillation. *Am J Med* **2015**, *128* (2), 192.e11-8.
8. Sangoi, M. B.; Da Silva, S. H.; da Silva, J. E.; Moresco, R. N., Relation between red blood cell distribution width and mortality after acute myocardial infarction. In *Int J Cardiol*, Netherlands, 2011; Vol. 146, pp 278-80.
9. Felker, G. M.; Allen, L. A.; Pocock, S. J.; Shaw, L. K.; McMurray, J. J.; Pfeffer, M. A.; Swedberg, K.; Wang, D.; Yusuf, S.; Michelson, E. L.; Granger, C. B., Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. In *J Am Coll Cardiol*, United States, 2007; Vol. 50, pp 40-7.
10. Ani, C.; Ovbiagele, B., Elevated red blood cell distribution width predicts mortality in persons with known stroke. *J Neurol Sci* **2009**, *277* (1-2), 103-8.
11. Hampole, C. V.; Mehrotra, A. K.; Thenappan, T.; Gomberg-Maitland, M.; Shah, S. J., Usefulness of red cell distribution width as a prognostic marker in pulmonary hypertension. *Am J Cardiol* **2009**, *104* (6), 868-72.
12. Ye, Z.; Smith, C.; Kullo, I. J., Usefulness of red cell distribution width to predict mortality in patients with peripheral artery disease. *Am J Cardiol* **2011**, *107* (8), 1241-5.
13. Kim, C. H.; Park, J. T.; Kim, E. J.; Han, J. H.; Han, J. S.; Choi, J. Y.; Han, S. H.; Yoo, T. H.; Kim, Y. S.; Kang, S. W.; Oh, H. J., An increase in red blood cell distribution width from baseline predicts mortality in patients with severe sepsis or septic shock. *Crit Care* **2013**, *17* (6), R282.
14. Bazick, H. S.; Chang, D.; Mahadevappa, K.; Gibbons, F. K.; Christopher, K. B., Red cell distribution width and all-cause mortality in critically ill patients. *Crit Care Med* **2011**, *39* (8), 1913-21.
15. Nada, A. M., Red cell distribution width in type 2 diabetic patients. *Diabetes Metab Syndr Obes* **2015**, *8*, 525-33.
16. Sánchez-Chaparro, M. A.; Calvo-Bonacho, E.; González-Quintela, A.; Cabrera, M.; Sáinz, J. C.; Fernández-Labandera, C.; Aguado, L. Q.; Meseguer, A. F.; Valdivielso, P.; Román-García, J.;

Ibermutuamur, C. R. A. S. G., Higher red blood cell distribution width is associated with the metabolic syndrome: results of the Ibermutuamur Cardiovascular Risk assessment study. In *Diabetes Care*, United States, 2010; Vol. 33, p e40.

17. White, W. B.; Cannon, C. P.; Heller, S. R.; Nissen, S. E.; Bergenstal, R. M.; Bakris, G. L.; Perez, A. T.; Fleck, P. R.; Mehta, C. R.; Kupfer, S.; Wilson, C.; Cushman, W. C.; Zannad, F., Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* **2013**, *369* (14), 1327-35.

18. White, W. B.; Bakris, G. L.; Bergenstal, R. M.; Cannon, C. P.; Cushman, W. C.; Fleck, P.; Heller, S.; Mehta, C.; Nissen, S. E.; Perez, A.; Wilson, C.; Zannad, F., EXamination of cArdiovascular outcoMes with alogliptIN versus standard of carE in patients with type 2 diabetes mellitus and acute coronary syndrome (EXAMINE): a cardiovascular safety study of the dipeptidyl peptidase 4 inhibitor alogliptin in patients with type 2 diabetes with acute coronary syndrome. *Am Heart J* **2011**, *162* (4), 620-626.e1.

19. Danese, E.; Lippi, G.; Montagnana, M., Red blood cell distribution width and cardiovascular diseases. *J Thorac Dis* **2015**, *7* (10), E402-11.

20. Ferreira, J. P.; Girerd, N.; Pellicori, P.; Duarte, K.; Girerd, S.; Pfeffer, M. A.; McMurray, J. J.; Pitt, B.; Dickstein, K.; Jacobs, L.; Staessen, J. A.; Butler, J.; Latini, R.; Masson, S.; Mebazaa, A.; Rocca, H. P.; Delles, C.; Heymans, S.; Sattar, N.; Jukema, J. W.; Cleland, J. G.; Zannad, F.; Rossignol, P., Renal function estimation and Cockcroft-Gault formulas for predicting cardiovascular mortality in population-based, cardiovascular risk, heart failure and post-myocardial infarction cohorts: The Heart 'OMics' in AGEing (HOMAGE) and the high-risk myocardial infarction database initiatives. *BMC Med* **2016**, *14* (1), 181.

21. Hwang, Y. C.; Morrow, D. A.; Cannon, C. P.; Liu, Y.; Bergenstal, R.; Heller, S.; Mehta, C.; Cushman, W.; Bakris, G. L.; Zannad, F.; White, W. B., High-sensitivity C-reactive protein, low-density lipoprotein cholesterol and cardiovascular outcomes in patients with type 2 diabetes in the EXAMINE (Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care) trial. *Diabetes Obes Metab* **2018**, *20* (3), 654-659.

22. White, W. B.; Jalil, F.; Cushman, W. C.; Bakris, G. L.; Bergenstal, R.; Heller, S. R.; Liu, Y.; Mehta, C.; Zannad, F.; Cannon, C. P., Average Clinician-Measured Blood Pressures and Cardiovascular Outcomes in Patients With Type 2 Diabetes Mellitus and Ischemic Heart Disease in the EXAMINE Trial. *J Am Heart Assoc* **2018**, *7* (20), e009114.

23. Cavender, M. A.; White, W. B.; Jarolim, P.; Bakris, G. L.; Cushman, W. C.; Kupfer, S.; Gao, Q.; Mehta, C. R.; Zannad, F.; Cannon, C. P.; Morrow, D. A., Serial Measurement of High-Sensitivity Troponin I and Cardiovascular Outcomes in Patients With Type 2 Diabetes Mellitus in the EXAMINE Trial (Examination of Cardiovascular Outcomes With Alogliptin Versus Standard of Care). *Circulation* **2017**, *135* (20), 1911-1921.

24. Ferreira, J. P.; Mehta, C.; Sharma, A.; Nissen, S. E.; Rossignol, P.; Zannad, F., Alogliptin after acute coronary syndrome in patients with type 2 diabetes: a renal function stratified analysis of the EXAMINE trial. *BMC Med* **2020**, *18* (1), 165.

25. Elharram, M.; Sharma, A.; White, W.; Bakris, G.; Rossignol, P.; Mehta, C.; Ferreira, J. P.; Zannad, F., Timing of randomization after an acute coronary syndrome in patients with type 2 diabetes mellitus. *Am Heart J* **2020**, *229*, 40-51.

26. Ferreira, J. P.; Sharma, A.; Mehta, C.; Bakris, G.; Rossignol, P.; White, W. B.; Zannad, F., Multi-proteomic approach to predict specific cardiovascular events in patients with diabetes and myocardial infarction: findings from the EXAMINE trial. *Clin Res Cardiol* **2020**.

27. Fine, JP; Gray, RJ. A proportional hazards model for the subdistribution of a competing risk. *J Am Stat Assoc* **1999**, *94* :496-509.

28. Lippi, G.; Targher, G.; Montagnana, M.; Salvagno, G. L.; Zoppini, G.; Guidi, G. C., Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. *Arch Pathol Lab Med* **2009**, *133* (4), 628-32.

29. Tonelli, M.; Sacks, F.; Arnold, M.; Moye, L.; Davis, B.; Pfeffer, M., Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. *Circulation* **2008**, *117* (2), 163-168.
30. Ramamurthy, V.; Yamniuk, A. P.; Lawrence, E. J.; Yong, W.; Schneeweis, L. A.; Cheng, L.; Murdock, M.; Corbett, M. J.; Doyle, M. L.; Sheriff, S., The structure of the death receptor 4-TNF-related apoptosis-inducing ligand (DR4-TRAIL) complex. *Acta Crystallogr F Struct Biol Commun* **2015**, *71* (Pt 10), 1273-81.
31. Hymowitz, S. G.; Christinger, H. W.; Fuh, G.; Ultsch, M.; O'Connell, M.; Kelley, R. F.; Ashkenazi, A.; de Vos, A. M., Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. *Mol Cell* **1999**, *4* (4), 563-71.
32. Skau, E.; Henriksen, E.; Wagner, P.; Hedberg, P.; Siegbahn, A.; Leppert, J., GDF-15 and TRAIL-R2 are powerful predictors of long-term mortality in patients with acute myocardial infarction. *Eur J Prev Cardiol* **2017**, *24* (15), 1576-1583.
33. Ridker, P. M.; Luscher, T. F., Anti-inflammatory therapies for cardiovascular disease. *Eur Heart J* **2014**, *35* (27), 1782-91.
34. Ferreira, J. P.; Verdonchot, J.; Collier, T.; Wang, P.; Pizard, A.; Bar, C.; Bjorkman, J.; Boccanelli, A.; Butler, J.; Clark, A.; Cleland, J. G.; Delles, C.; Diez, J.; Girerd, N.; Gonzalez, A.; Hazebroek, M.; Huby, A. C.; Jukema, W.; Latini, R.; Leenders, J.; Levy, D.; Mebazaa, A.; Mischak, H.; Pinet, F.; Rossignol, P.; Sattar, N.; Sever, P.; Staessen, J. A.; Thum, T.; Vodovar, N.; Zhang, Z. Y.; Heymans, S.; Zannad, F., Proteomic Bioprofiles and Mechanistic Pathways of Progression to Heart Failure. *Circ Heart Fail* **2019**, *12* (5), e005897.
35. Panwar, B.; Judd, S. E.; Wadley, V. G.; Jenny, N. S.; Howard, V. J.; Safford, M. M.; Gutierrez, O. M., Association of Fibroblast Growth Factor 23 With Risk of Incident Coronary Heart Disease in Community-Living Adults. *JAMA Cardiol* **2018**, *3* (4), 318-325.
36. Hao, H.; Li, X.; Li, Q.; Lin, H.; Chen, Z.; Xie, J.; Xuan, W.; Liao, W.; Bin, J.; Huang, X.; Kitakaze, M.; Liao, Y., FGF23 promotes myocardial fibrosis in mice through activation of beta-catenin. *Oncotarget* **2016**, *7* (40), 64649-64664.
37. He, X. W.; Li, W. L.; Li, C.; Liu, P.; Shen, Y. G.; Zhu, M.; Jin, X. P., Serum levels of galectin-1, galectin-3, and galectin-9 are associated with large artery atherosclerotic stroke. *Sci Rep* **2017**, *7*, 40994.
38. Nguyen, Q. P.; Deng, T. Z.; Witherden, D. A.; Goldrath, A. W., Origins of CD4(+) circulating and tissue-resident memory T-cells. *Immunology* **2019**, *157* (1), 3-12.
39. Nishikimi, T.; Nakagawa, Y., Adrenomedullin as a Biomarker of Heart Failure. *Heart Fail Clin* **2018**, *14* (1), 49-55.
40. Voors, A. A.; Kremer, D.; Geven, C.; Ter Maaten, J. M.; Struck, J.; Bergmann, A.; Pickkers, P.; Metra, M.; Mebazaa, A.; Düngen, H. D.; Butler, J., Adrenomedullin in heart failure: pathophysiology and therapeutic application. *Eur J Heart Fail* **2019**, *21* (2), 163-171.
41. Packer, M., Mechanisms Leading to Differential Hypoxia Inducible Factor Signaling in the Diabetic Kidney: Modulation by SGLT2 Inhibitors and Hypoxia Mimetics. *Am J Kidney Dis* **2020**.

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

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

Alogliptin allocation	2073 (50.6%)	628 (49.0%)	0.32
-----------------------	--------------	-------------	------

Legend: RDW, red cell distribution width; BMI, body mass index; HF, heart failure; PAD, peripheral artery disease; eGFR, estimated glomerular filtration rate; hsTnl, 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

Variable	Rho	P-value
Hemoglobin	-0.31	<0.001
TRAILR2	0.26	<0.001
FGF23	0.24	<0.001
TNFRSF11A	0.23	<0.001
TNFRSF10A	0.22	<0.001
GAL9	0.22	<0.001
SPON2	0.22	<0.001
BNP	0.22	<0.001
CD4	0.22	<0.001
ADM	0.20	<0.001

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

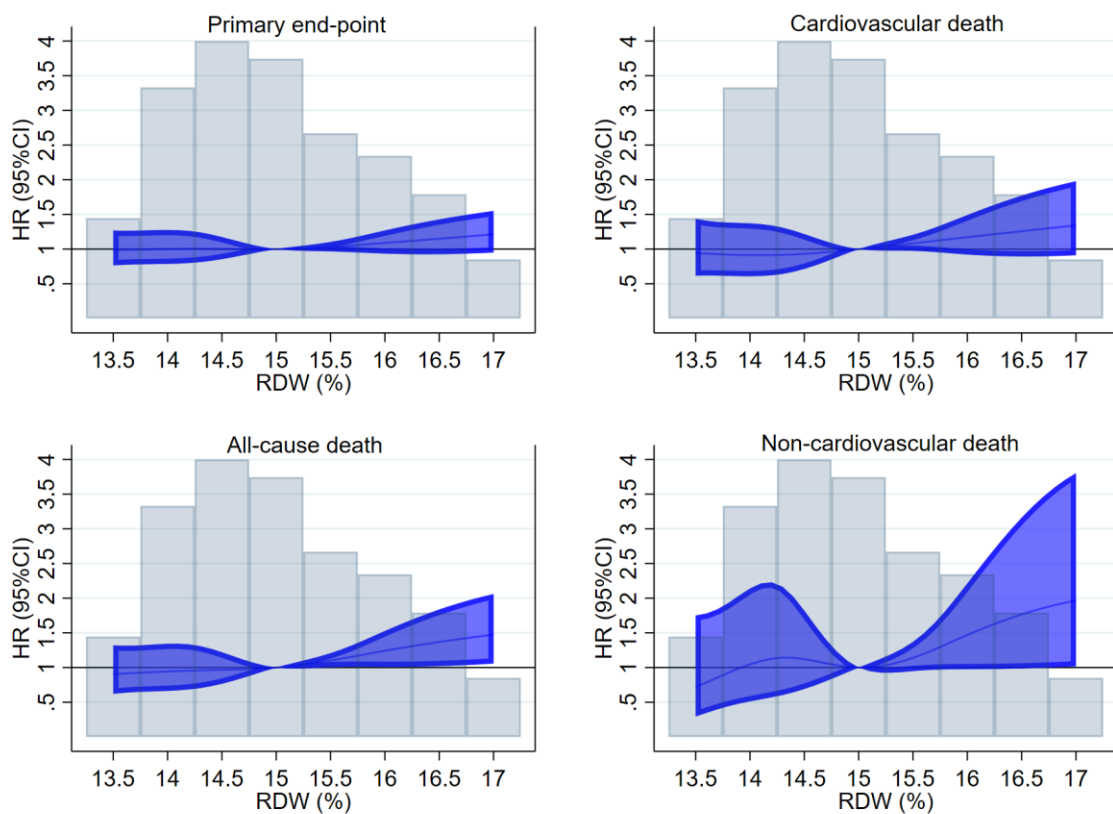
Legend: TRAILR2, TRAIL receptor 2 or death receptor 5; FGF23, fibroblast growth factor 23; TNFRSF11A, tumor necrosis factor receptor soluble fraction 11A; TNFRSF10A, tumor necrosis factor receptor soluble fraction 10A; Gal9, galectin 9; SPON2, spondin-2; BNP, brain natriuretic peptide; CD4, cluster of differentiation 4; ADM, adrenomedullin.

Table 3. Outcome associations of baseline and time-updated RDW categories (>16.1% vs. ≤16.1%)

End-point	RDW		Baseline RDW		P-value	Time-updated RDW		P-value
	≤16.1%	>16.1%	Crude model	Adj. model		Crude model	Adj. model	
	n =4098	n =1281	HR (95%CI)	HR (95%CI)		HR (95%CI)	HR (95%CI)	
Primary end-point	410 (10.0%)	211 (16.5%)	1.68 (1.43-1.99)	1.24 (1.04-1.49)	0.015	1.68 (1.43-1.97)	1.36 (1.16-1.61)	<0.001
All-cause death	194 (4.7%)	132 (10.3%)	2.18 (1.75-2.72)	1.48 (1.17-1.88)	0.001	2.60 (2.09-3.23)	2.01 (1.60-2.53)	<0.001
CV death	145 (3.5%)	97 (7.6%)	2.15 (1.66-2.77)	1.43 (1.09-1.88)	0.01	2.43 (1.89-3.13)	1.84 (1.41-2.40)	<0.001
Myocardial infarction	253 (6.2%)	107 (8.3%)	1.38 (1.10-1.73)	1.05 (0.83-1.34)	0.68	1.42 (1.14-1.76)	1.17 (0.94-1.47)	0.16
Stroke	40 (1.0%)	21 (1.6%)	1.70 (1.00-2.89)	1.51 (0.86-2.65)	0.15	0.93 (0.53-1.62)	0.82 (0.46-1.44)	0.48
HF hosp.	108 (2.6%)	87 (6.8%)	2.64 (1.99-3.51)	1.55 (1.15-2.10)	0.004	2.73 (2.06-3.62)	1.86 (1.39-2.50)	<0.001
CV death or HF hosp.	236 (5.8%)	166 (12.9%)	2.31 (1.89-2.81)	1.49 (1.21-1.84)	<0.001	2.57 (2.12-3.13)	1.91 (1.56-2.34)	<0.001
CV hosp.	561 (13.7%)	262 (20.4%)	1.54 (1.33-1.79)	1.23 (1.06-1.44)	0.008	1.50 (1.31-1.73)	1.30 (1.12-1.49)	0.001
CV death or CV hosp.	624 (15.2%)	300 (23.4%)	1.59 (1.38-1.82)	1.24 (1.07-1.43)	0.004	1.63 (1.43-1.86)	1.38 (1.20-1.58)	<0.001
Non-CV death	49 (1.2%)	35 (2.7%)	2.29 (1.48-3.53)	1.70 (1.08-2.68)	0.023	3.15 (2.04-4.86)	2.67 (1.72-4.15)	<0.001

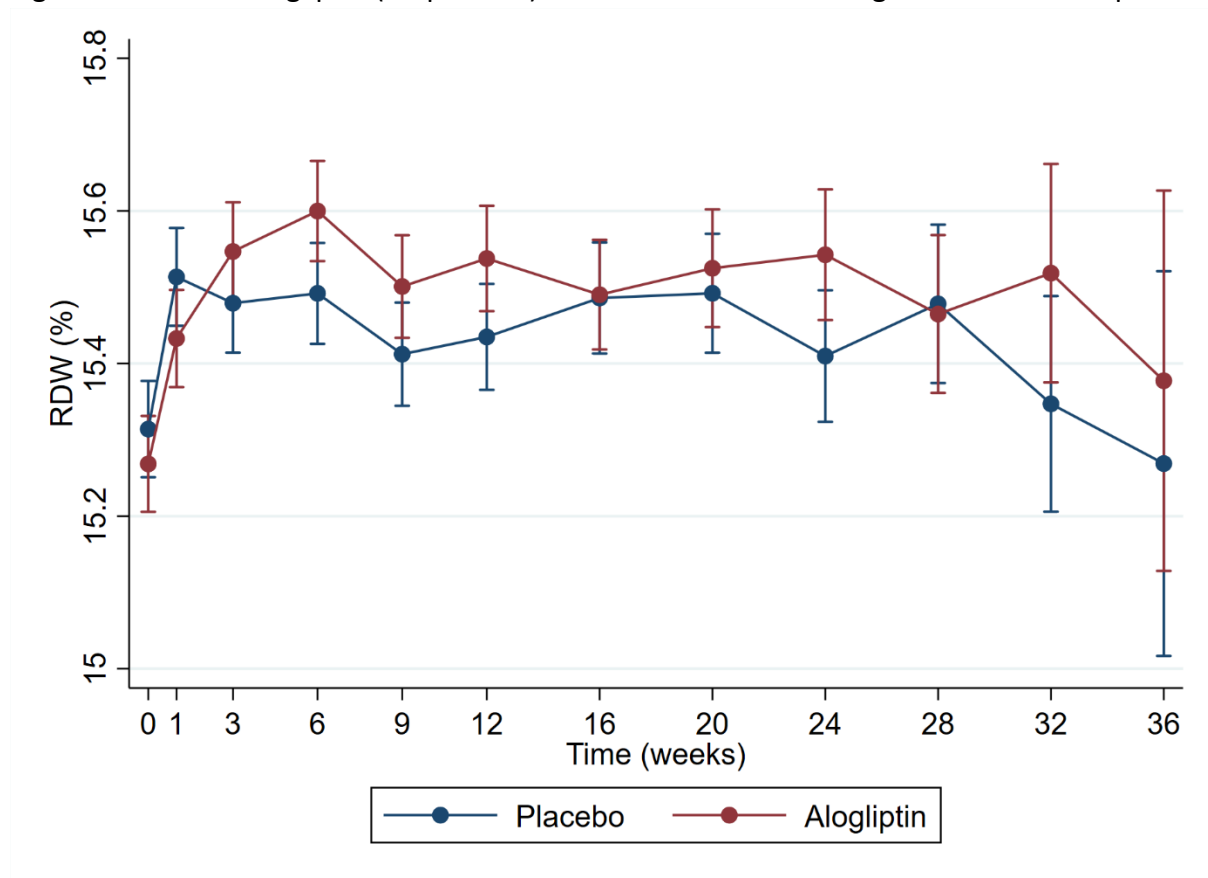
Legend: CV, cardiovascular; HF, heart failure; Hosp., hospitalization; Adj., adjusted on age, sex, smoking, diabetes duration, previous MI, previous HF hospitalization, previous stroke, atrial fibrillation, hypertension, systolic blood pressure, statin therapy, estimated glomerular filtration rate, anemia 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.