The Value of Red Cell Distribution Width as a Predictor for Prognosis Following Amputation in Diabetic Foot

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Sincerely,

Hang Hwan Cho

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ABSTRACT

The Value of Red Cell Distribution Width as a Predictor for Prognosis Following Amputation in Diabetic Foot

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Red cell distribution width (RDW) reflects the degree of heterogeneity of erythrocyte volume. The number of studies investigating the relationship between RDW and various human disorders has exponentially increased over the past decades. However, the association of RDW with diabetic foot amputation has not been evaluated to date. In this study, we assessed the value of RDW as a prognostic factor in diabetic foot amputation. Data on 415 patients with diabetic foot who underwent amputation between January 2009 and January 2019 were analyzed retrospectively. After establishing optimal cut-off point of preoperative RDW for all-cause mortality, univariable and multivariable analyses with Cox proportional hazard model for survivorship and logistic regression for length of hospital stay more than 30 days were performed to identify significant prognostic factors including RDW, other laboratory results, demographic variables and co-morbidities. RDW cut-off of 14.5% was found to be significantly associated with all-cause mortality (< 0.001). High RDW was a significant risk factor or all-cause mortality (hazard ratio[HR]: 2.42, 95% confidence interval [CI]: 1.46 to 4.00) on multivariable-adjusted regression analysis. High RDW was also associated with longer hospitalization (odds ratio: 2.17, 95% CI: 1.29 to

3.66). So high RDW over 14.5% value is an independent prognostic factor with increased mortality and prolonged hospital stay, implying that RDW may be a simple and inexpensive laboratory parameter for risk stratification in diabetic foot amputation.

Keywords: diabetic foot, amputation, red cell distribution width, mortality, length of hospital stay

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I. INTRODUCTION

The impact of the global burden of diabetes has been increasing over time. There is a report that the number of diabetic patients worldwide is expected to increase by 51% from 463 million to 700 million by 2045[1]. The longer the duration of diabetes, the higher risk of developing diabetic foot complications including ulcers or gangrene[2] Therefore, the explosive increase in diabetes will further attention to diabetic foot in the future. Diabetic foot ulcers (DFUs) that do not respond to proper conservative management is eventually forced to undergo amputation, which have still been performed every 30 seconds on the earth[3]. The 5-year survival rate of diabetic foot is comparable to that of malignant tumors, serving as a heavy medical and social burden[4].

As diabetic patients have multiple comorbidities or complications, their clinical assessment is difficult. In case of diabetic foot, neuropathy, vasculopathy, and other underlying condition are associated with complex interaction. The neuropathy causes

protective sensory deterioration, deformity by impaired motor function, and autonomic dysfunction. Peripheral vascular calcification with diabetes leads to insufficient oxygen supply to tissue. In addition, impaired vision due to diabetic retinopathy, decreased activity of daily living due to hemodialysis or other accompanying diseases deprives them of their ability to manage their own feet. Under these clinical setting, multidisciplinary team approach is proposed and applied as a management of diabetic patient.

For this reason, factors that can predict the prognosis of diabetic foot disease are needed, and these factors should not only be medically easy to detect, but also should not be an economic burden to the patient. Considering this aspect, one of the factors that can be used appropriately is the red cell distribution width (RDW).

RDW, indicating the heterogeneity of erythrocyte volume, has been used in the differential diagnosis of anemia so far[5]. In the recent decades, on the other hand, the investigations about the relationship between RDW and other disorders have been on the rise in past decades[5]. The clinical usefulness of RDW as a prognostic marker has been proven in cardiovascular disease[6], cancer[7,8], kidney disease[9,10], diabetes[11], and etc. However, the clinical role of RDW in mortality and length of hospitalization of diabetic foot amputation has not been evaluated enough up to date. This study aimed to investigate the value of RDW as a prognostic parameter in diabetic foot amputation.

II. MATERIALS AND METHODS

1. Patients and Study Design

This was a single-institution study approved by our Institutional Review to perform a retrospective cohort analysis of patients with DFUs who underwent lower extremity amputation (LEA) between January 2009 and January 2019. During the study period, LEA was performed on 444 patients with diabetes mellitus. LEA was indicated on wet gangrene that did not recover despite of aggressive conservative treatment, dry gangrene combined with peripheral arterial disease, osteomyelitis, severe pain disturbing activity of daily living, and failed reconstruction of Charcot arthropathy. Patients with traumatic LEA, soft tissue malignant tumor were excluded and patients with a history of LEA within 1 year were also excluded for diminish the carryover effects.

2. Preoperative Evaluation and Preparation

Electronic medical records and databases were reviewed for patients eligible for the present study. Demographic information of the study population including age, sex, and body mass index (BMI), smoking at the time of the amputation were collected.

Prior to surgery, all patients were coordinated to confirm the operability of each clinician based on the underlying co-morbidities and basic preoperative examination including laboratory results, chest radiograph, electrocardiography and further evaluation as request of the clinician. Hypertension(HTN), coronary artery disease(CAD), old cerebrovascular accident(CVA), obstructive pulmonary disease(COPD), solid organ (liver or kidney) transplantation, and end-stage renal disease(ESRD) on hemodialysis(HD) or peritoneal dialysis(PD) were assessed as underlying disease. Hemoglobin (Hb, g/dL), red blood cell distribution width (RDW, %), white blood cell (WBC, $\times 10^3/\mu L$) count, lymphocyte count ($\times 10^3/\mu L$), erythrocyte sedimentation rate (ESR, mm/hr), C-reactive protein (CRP, mg/L),

glycated hemoglobin (HbA1c, %), albumin (g/dL), and estimates glomerular filtration rate (eGFR) by modification of diet in renal disease (mL/min/1.73m^2, MDRD) equation were collected in the preoperative laboratory results. If more than examination results were available within 60 days preoperatively, the result closest to the date of surgery was used. BMI was divided into normal and obese groups based on 25kg/m^2. The evaluation of renal function and kidney disease were divided into three groups based on the eGFR and dialysis; normal over 60mL/min/1.73m^2, chronic kidney disease over 15 and less than 60mL/min/1.73m^2, and end-stage renal disease (ESRD) under 15mL/min/1.73m^2 with dialysis.

3. Lower Extremity Amputation, Postoperative Management and Discharge

All LEAs were performed under general, spinal, or regional anesthesia. The amputation level was determined while maintaining the greatest residual limb length but removing all unviable or infected tissue and securing sufficient soft tissue coverage. An amputation higher than the level of the ankle joint was regarded as a major amputation, while any amputations below the ankle joint were viewed as minor amputations. After the amputation, the stump wound was managed by performing a daily sterile compression dressing. If there were no signs of stump infection and stability of the wound healing was confirmed, the discharge of the patient was planned. Through multidisciplinary team approach, discharge from hospital was confirmed based on the patient's medical history and current status with consultation of each clinician.

4. Primary and Secondary Endpoints

The primary endpoint was mortality. The survival period was calculated based on the last visit date of the hospital and the death was confirmed on the electronic medical records.

The secondary endpoint was prolonged length of hospital stay (LOS) more than 30 days after amputation. The LOS was defined as the duration from the day of amputation to discharge.

5. Statistical Analysis

Patient characteristics and clinical data are presented as mean ± standard deviation or count (percentage). To obtain the optimal cut-off value of RDW related to all-cause mortality, we used the maximum choice log-rank test for survival analysis and receiver operating characteristic (ROC) curve analysis to obtain the optimal cut-off value of RDW related to prolonged LOS. Cox proportional hazard and logistic regression models were used to assess significant prognostic variables associated with the mortality and LOS. A P value < 0.05 was considered statistically significant. Factors that were significant ($P < 0.05$) in univariate analysis were entered into a multivariate Cox and logistic regression analysis. All statistical analyses were performed using R (version 4.0.0, R Foundation for Statistical Computing).

III. RESULTS

1. Patient Demographics and Baseline Characteristics

Of the 444 patients initially identified, 29 patients were excluded. 2 patients underwent LEA for trauma, 27 patients were excluded for washout period within 1 year of previous LEA, and there was no case of malignant soft tissue tumor. The final cohort included 415 patients.

Patient characteristics based on clinical information and preoperative laboratory results are presented in Table 1. The mean follow-up period after LEA was 3.12 ± 2.85 years. The mean age of cohort was 64.13 ± 11.86 years and 75.66% of patients were male. HTN was the most frequent combined underlying disease, and followed by CAD, and kidney disease. The mean preoperative Hb was 10.50 ± 1.78 g/dL, RDW was 14.29 ± 1.80 %, and HbA1c was 7.94 ± 1.88. Minor amputation accounted for 88.92% of all LEA and remaining 11.08% were major amputation.

Table 1. Baseline Characteristics of clinical information and preoperative laboratory results

Category	subgroup		n (%)
Age (years)			64.13 ± 11.86
Sex	Female		101 (24.34%)
	Male		314 (75.66%)
BMI (kg/m2)			23.13 ± 3.39
	< 25		306 (73.73%)
	≥ 25		109 (26.27%)
Smoking	Ex-smoker		135 (32.53%)
	Current smoker		57 (13.73%)
Co-morbidities	HTN		320 (77.11%)
	CAD		118 (28.43%)
	Old CVA		64 (15.42%)
	COPD		37 (8.92%)
	Transplantation		39 (9.40%)
	Kidney disease	CKD (15≤eGFR<60, mL/min/1.73 m²)	115 (27.71%)
		ESRD on HD/PD (eGFR<15, mL/min/1.73 m²)	97 (23.37%)
Preop. Lab.	Hb (g/dL)		10.5 ± 1.8
	RDW (%)		14.3 ± 1.8
		< 14.5	268 (64.58%)
		≥ 14.5	147 (35.42%)
	WBC (×103/μL)		11.36 ± 5.74
	Lymphocyte (×103/μL)		1.42 ± 0.57
	ESR (mm/hr)		85.39 ± 30.87

	CRP (mg/L)	77.91 ± 82.94
	HbA1c (%)	7.94 ± 1.9
	Albumin (g/dL)	3.1 ± 0.6
Level of amputation	Minor	369 (88.92%)
	Major	46 (11.08%)
Mean Follow-up Period (years)		3.12 ± 2.85

BMI body mass index, HTN hypertension, CAD coronary artery disease, CVA cerebrovascular accident, COPD chronic obstructive pulmonary disease, CKD chronic kidney disease, eGFR estimated glomerular filtration rate, ESRD end stage renal disease , HD hemodialysis, PD peritoneal dialysis, Hb hemoglobin, RDW red cell distribution width, WBC white blood cell, ESR erythrocyte sedimentation rate, CRP C-reactive protein, HbA1c glycated hemoglobin

2. Primary and Secondary Endpoints

The overall estimated survival rate of the entire cohort was as follows: 1 year-89.4%, 3 years-82.8%, 5 years-75.7%, and 7 years-68.7%. Based on the maximally selected rank statistics, the optimal cut-off point for preoperative RDW to mortality was 14.5% (Figure 1).

Using the ROC analysis, RDW was found to be a significant predictor of prolonged LOS after LEA (Figure 2; area under the curve AUC = 0.619, 95% confidence interval CI 0.554 – 0.683, $P < 0.001$) with cut-off of RDW = 14.5% (sensitivity 53.1%, specificity 67.1%).

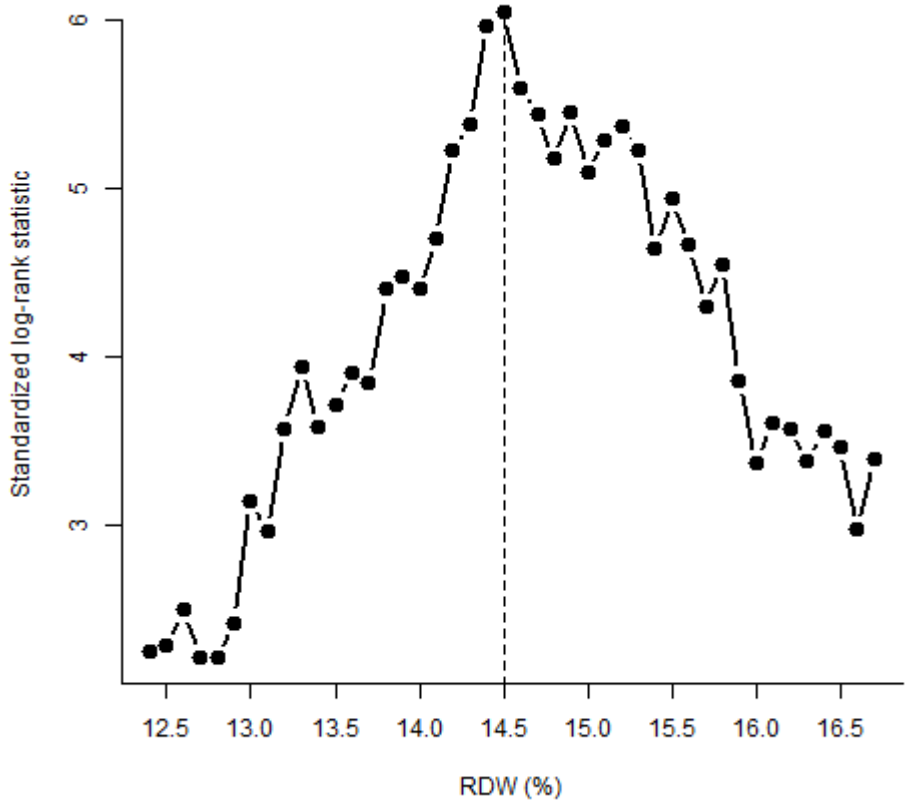

Figure 1. Maximally selected rank statistics. In the mortality analysis after lower extremity amputation (LEA), the cut-off value of RDW obtained by maximally selected logrank statistics using the maxstat package of R, a statistical program, was measured as 14.5%.

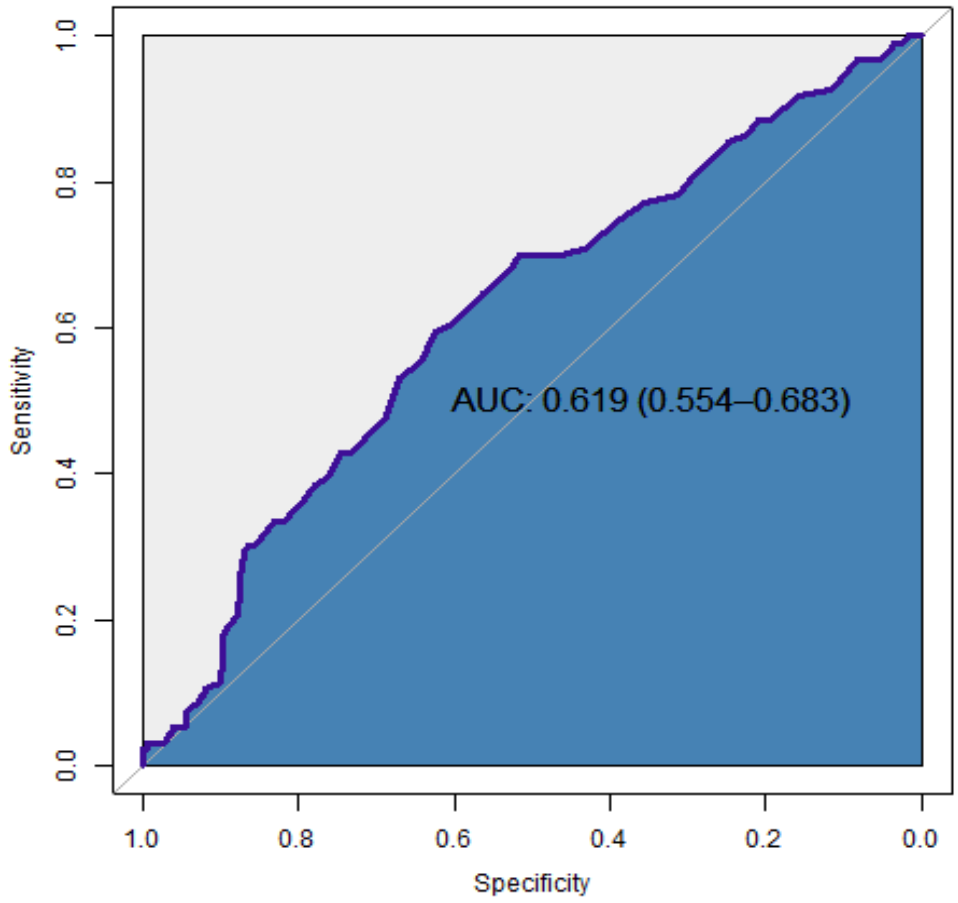

Figure 2. ROC curve. Prolonged length of hospital stay (LOS) analysis was performed using the receiver operating characteristic (ROC) curve, and the cut-off value of RDW when area under the curve (AUC) was 0.619, sensitivity was 53.1%, and specificity was 67.1% was measured as 14.5%.

Univariable and multivariable Cox proportional hazard regression analysis are shown in Table 2 and Table 3.

In univariate regression, statistically significant factors related to mortality after LEA were RDW(the hazard ratio HR, 1.35; 95% CI, 1.23 – 1.49; P = <0.001, ≥ 14.5%, HR, 3.74; 95% CI, 2.39 – 5.83; P = <0.001), age(HR, 1.06; 95% CI, 1.03 – 1.08; P = <0.001), HTN(HR, 2.83; 95% CI, 1.36 – 5.86; P = 0.005), CAD(HR, 1.95; 95% CI, 1.26 – 3.00; P = 0.003), old CVA(HR, 2.36; 95% CI, 1.44 – 3.85; P = 0.001), ESRD(HR, 3.51; 95% CI, 2.05 – 6.01; P = <0.001), Hb(HR, 0.82; 95% CI, 0.72 – 0.93; P = 0.002), WBC(HR, 0.95; 95% CI, 0.91 – 1.00; P = 0.038), and lymphocyte(HR, 0.66; 95% CI, 0.43 – 1.00; P = 0.048). On the other hand, HbA1c (HR, 0.92; 95% CI, 0.81 – 1.05; P = 0.205) and level of amputation(HR, 1.58; 95% CI, 0.84 – 2.99; P = 0.156) did not correlate with mortality after LEA(Table 2).

After adjusting for variates which were significant at univariable regression, high preoperative RDW levels over 14.5% were significantly associated with all-cause mortality after diabetic foot amputation (adjusted HR, 2.55; 95% CI, 1.55 – 4.19; $P < 0.001$)(Table 3). Other variables positively associated with mortality were age (adjusted HR, 1.06; 95% CI, 1.04 – 1.08; $P < 0.001$) and ESRD (adjusted HR, 2.29; 95% CI, 1.27 – 4.11; $P = 0.006$)(Table 3).

Table 2. Univariable Cox proportional hazard model

Variables		HR (95% CI)	P value
Age (years)		1.06 (1.03 – 1.08)	< 0.001
Sex	Female		
	Male	0.91 (0.56 – 1.47)	0.704
BMI (kg/m2)	< 25		
	≥ 25	0.68 (0.40 – 1.14)	0.145
Smoking	Nonsmoker		
	Exsmoker	0.76(0.49 – 1.19)	0.234
	Smoker	0.44(0.19 – 1.02)	0.054
HTN		2.83 (1.36 – 5.86)	0.005
CAD		1.95 (1.26 – 3.00)	0.003
Old CVA		2.36 (1.44 – 3.85)	0.001
COPD		0.50 (0.18 – 1.37)	0.180
Transplantation		0.90 (0.45 – 1.79)	0.757
Kidney disease	≥ 60		
(eGFR by MDRD,	15 ≤ CKD < 60	1.99 (1.15 – 3.34)	0.014
mL/min/1.73m²)	15≥ESRD	3.51 (2.05 – 6.01)	< 0.001
Hb (g/dL)		0.82 (0.72 – 0.93)	0.002
RDW (%)		1.35 (1.23 – 1.49)	< 0.001
	< 14.5		
	≥ 14.5	3.74 (2.39 – 5.83)	< 0.001
WBC (×103/μL)		0.95 (0.91 – 1.00)	0.038
Lymphocyte (×103/μL)		0.66 (0.43 – 1.00)	0.048
ESR (mm/hr)		1.00 (0.99 – 1.01)	0.951
CRP (mg/L)		1.00 (1.00 – 1.00)	0.383
HbA1c (%)		0.92 (0.81 – 1.05)	0.205
Albumin (g/dL)		0.76 (0.54 – 1.06)	0.103

Level of Amputation	Minor		
	Major	1.58 (0.84 – 2.99)	0.156

Among the all-cause mortality analysis using Cox proportional hazard regression analysis after lower extremity amputation (LEA), as a result of univariate Cox proportional hazard regression analysis, age, HTN, CAD, old CVA, CKD, ESRD, Hb, RDW, WBC, and lymphocyte were statistically significant. The hazard ratio was high at 3.74 in the group with RDW ≥ 14.5%.

Table 3. Multivariable Cox proportional hazard model

Variables		RDW(%) < 14.5		RDW(%) ≥ 14.5	
		HR (95% CI)	P value	HR (95% CI)	P value
Age (years)		1.07 (1.04 – 1.09)	< 0.001	1.06 (1.04 – 1.08)	< 0.001
HTN		1.42 (0.66 – 3.05)	0.373	1.60 (0.75 – 3.44)	0.227
CAD		1.22 (0.76 – 1.95)	0.408	1.21 (0.76 – 1.93)	0.412
Old CVA		1.57 (0.93 – 1.57)	0.093	1.42 (0.84 – 2.40)	0.196
eGFR	15 ≤ CKD < 60	1.48 (0.84 – 2.60)	0.172	1.51 (0.86 – 2.67)	0.154
(MDRD, mL/min/1.73 m²)	ESRD < 15	2.37 (1.32 – 4.23)	0.004	2.29 (1.27 – 4.11)	0.006
Hb (g/dL)		0.94 (0.82 – 1.08)	0.388	0.95 (0.82 – 1.09)	0.456
RDW (%)		1.29 (1.15 – 1.44)	< 0.001	2.55 (1.55 – 4.19)	< 0.001
WBC (×103/μL)		0.99 (0.94 – 1.03)	0.554	0.99 (0.94 – 1.03)	0.572
Lymphocyte (×103/μL)		0.68 (0.43 – 1.07)	0.094	0.68 (0.44 – 1.06)	0.087

Among the all-cause mortality analysis after lower extremity amputation (LEA), after adjusting the statistically significant factors in the univariate cox regression analysis, multivariate cox regression was performed, and statistically significant predictors in the multivariate cox regression were identified as age, ESRD, and RDW. The hazard ratio was high at 2.55 in the group with RDW ≥ 14.5%.

For every 1 percentage point increase in RDW, risk of death increased by 29%. The cumulative survival curves showed superior survivorship after LEA with low preoperative RDW (< 14.5%) group compared with a high preoperative RDW (≥ 14.5%) group (P < 0.001) (Figure 3).

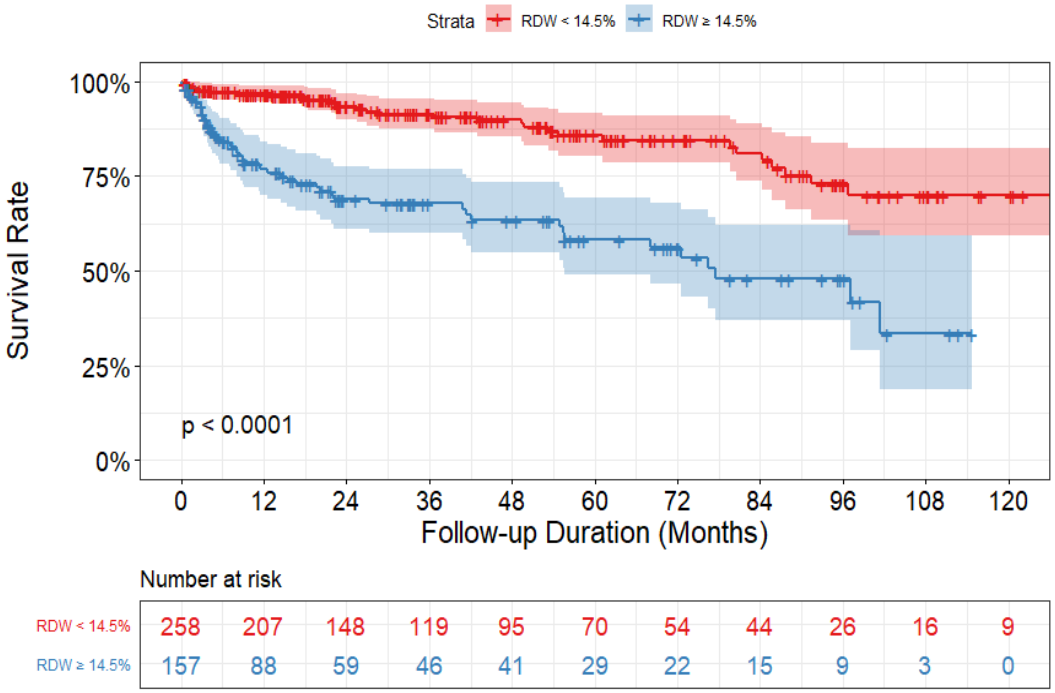

Figure 3. Log-rank test. Based on the RDW cut-off value of 14.5%, the group was divided into two groups: a group with an RDW of 14.5% or more and a group with an RDW of less than 14.5%, and survival analysis was performed for 120 months using the Log-rank test. As a result, the survival rate was superior in the group with RDW less than 14.5%.

The mean LOS was 24.62 ± 24.70 days. Univariable and multivariable logistic regression analysis are shown in Table 4 and Table 5.

In univariate regression, statistically significant factors related to prolonged LOS after LEA were RDW(HR, 1.23; 95% CI, 1.09 – 1.39; P = <0.001, ≥ 14.5%, HR, 2.31; 95% CI, 1.45 – 3.68; P = <0.001), ESRD(HR, 2.06; 95% CI, 1.18 – 3.58; P = 0.011), Hb(HR, 0.80; 95% CI, 0.70 – 0.92; P = 0.002), lymphocyte(HR, 0.49; 95% CI, 0.31 – 0.79; P = 0.003), ESR(HR, 1.01; 95% CI, 1.00 – 1.02; P = 0.025) and CRP(HR, 1.00; 95% CI, 1.00 – 1.01; P = 0.018). On the other hand, HbA1c (HR, 1.03; 95% CI, 0.92 – 1.16; P = 0.609) and level of amputation(HR, 1.61; 95% CI, 0.82 – 3.18; P = 0.170) did not correlate with prolonged LOS after LEA(Table 4).

Multivariable logistic regression analysis revealed that high preoperative RDW levels over 14.5% was the only associated variable for longer hospital stay more than 30 days after adjustment (adjusted odds ratio OR, 2.17; 95% CI, 1.29 – 3.66; *P* = 0.004)(Table 5).

Table 4. Univariable logistic regression model

Variables		OR (95% CI)	P value
Age (years)		0.99 (0.98 – 1.01)	0.549
Sex	Female		
	Male	0.76 (0.46 – 1.28)	0.306
BMI (kg/m2)	< 25		
	≥ 25	0.66 (0.38 – 1.15)	0.142
Smoking	Nonsmoker		
	Exsmoker	0.68 (0.41 – 1.14)	0.141
	Smoker	0.97 (0.50 – 1.88)	0.929
HTN		0.93 (0.54 – 1.60)	0.795
CAD		1.50 (0.92 – 2.45)	0.106
Old CVA		0.97 (0.51 – 1.84)	0.917
COPD		1.65 (0.79 – 3.42)	0.181
Transplantation		1.01 (0.46 – 2.22)	0.974
Kidney disease	≥ 60		
(eGFR by MDRD,	15 ≤ CKD < 60	1.25 (0.71 – 2.19)	0.437
mL/min/1.73m²)	ESRD	2.06 (1.18 – 3.58)	0.011
Hb (g/dL)		0.80 (0.70 – 0.92)	0.002
RDW (%)		1.23 (1.09 – 1.39)	0.001
	< 14.5		
	≥ 14.5	2.31 (1.45 – 3.68)	< 0.001
WBC (×103/μL)		1.02 (0.98 – 1.06)	0.25
Lymphocyte (×103/μL)		0.49 (0.31 – 0.79)	0.003
ESR (mm/hr)		1.01 (1.00 – 1.02)	0.025
CRP (mg/L)		1.00 (1.00 – 1.01)	0.018
HbA1c (%)		1.03 (0.92 – 1.16)	0.609

Albumin (g/dL)		0.75 (0.52 – 1.07)	0.108
Level of Amputation	Minor		
	Major	1.61 (0.82 – 3.18)	0.170

Among the prolonged length of hospital stay (LOS) analysis using logistic regression analysis after lower extremity amputation (LEA), as a result of univariate logistic regression analysis, ESRD, Hb, RDW, WBC, lymphocyte, ESR, and CRP were statistically significant. The hazard ratio was high at 2.31 in the group with RDW ≥ 14.5%

Table 5. Multivariable logistic regression model

Variables		RDW(%) < 14.5		RDW(%) ≥ 14.5	
		HR (95% CI)	P value	HR (95% CI)	P value
eGFR (MDRD, mL/min/ 1.73 m²)	15 ≤ CKD < 60	1.04 (0.57 – 1.90)	0.889	1.04 (0.57 – 1.90)	0.895
	ESRD < 15	1.39 (0.76 – 2.54)	0.286	1.32 (0.72 – 2.43)	0.375
Hb		0.90 (0.76 – 1.05)	0.171	0.90 (0.77 – 1.05)	0.191
RDW (%)		1.20 (1.05 – 1.37)	0.008	2.17 (1.29 – 3.66)	0.004
Lymphocyte		0.66 (0.41 – 1.07)	0.093	0.64 (0.39 – 1.03)	0.066
ESR		1.00 (1.00 – 1.01)	0.384	1.01 (1.00 – 1.01)	0.310
CRP		1.00 (1.00 – 1.01)	0.063	1.00 (1.00 – 1.01)	0.074

Among the prolonged length of hospital stay (LOS) analysis after lower extremity amputation (LEA), multivariate logistic regression was performed after adjusting statistically significant factors in univariate logistic regression analysis, and RDW was the only statistically significant predictor in multivariate logistic regression. The hazard ratio was high at 2.17 in the group with RDW ≥ 14.5%.

In the analysis of RDW as a continuous variable, for each 1 percentage point increase in RDW, there was a 20% increase in LOS over 30 days.

IV. DISCUSSION

Most diabetic amputee patients do not have enough financial space, so the treatment cost itself can be a huge burden on the patient. Therefore, the significance of this study can be said to be to find prognostic factors that can be easily confirmed medically, such as basic tests performed in diabetic foot disease, and that do not impose an economic burden on the patient. In that sense, the RDW test can be viewed as a suitable test that satisfies both factors. Therefore, this study is different from other studies in that it considers not only mortality but also the LOS in determining the prognosis of foot amputees according to the RDW level for diabetic foot disease patients treated at a tertiary medical institution called a university hospital. The results of this study showed that high RDW values had a statistically significant relationship with both mortality and LOS even after adjusting for confounding variables. Also, the practicality of the RDW value was confirmed by finding the cut-off value of the appropriate RDW value. The present study is the first to evaluate the impact of RDW on diabetic foot patient survivorship and LOS. In this analysis, we identified that preoperative RDW over 14.5% was not only associated with high mortality but also with prolonged LOS in diabetic foot amputation.

Increased RDW values have also been associated with diabetes-associated complications. H. Atalay et al. said that low RDW values were strongly associated with diabetic ketoacidosis (DKA), and that the RDW/MCV ratio alone reflects DKA markedly stronger than both RDW and MCV values[12]. And Min Zhang et al. reported that RDW is related to microalbuminuria in patients with type 2 diabetes mellitus and also to diabetes nephropathy[13]. In a research article conducted in 2021, Yingbo Ma et al. reported significantly increased RDW in diabetic retinopathy patients, and confirmed that increased RDW is an independent risk factor for diabetic retinopathy. Also, RDW could be a simple, inexpensive, and reliable parameter for judging the prognosis of diabetic retinopathy[14].

In addition, other studies on the relationship between DFUs and RDW, one of the fatal complications of diabetes, have shown that an increase in RDW has a significant effect on the prognosis of DFUs. Arıcan G et al. reported that in patients with RDW > 13.4% and DFUs, major amputation found to be significant[15], Hong J et al. reported that two factors, RDW and RDW/ALB ratio, are independent prognostic indicators of mortality in DFUs. In particular, the RDW/ALB ratio was said to be superior to RDW in prognostic judgment in mild rather than severe patients[16].

Age, ESRD, HbA1c and major amputation are known prognostic factors after diabetic foot amputation. Age[17,18] and ESRD[19,20,21] was found to be independent variables associated mortality after diabetic foot LEA in our study. However, HbA1c and major amputation was not a significant prognostic factor in our study.

HbA1c is more commonly used than RDW, and is an economical and easy-to-implement test that is widely known as an important indicator for determining whether or not HbA1c patients are controlled in the mid- to long-term. Therefore, if HbA1c is as statistically significant as RDW as a prognostic factor in this study, it was thought that it would have important value as an auxiliary indicator for determining the prognosis after LEA together with RDW. Therefore, in our study, we paid attention to the results of HbA1c as well as RDW, In the univariable Cox proportional hazard regression analysis of LEA patients, the HR (95% CI) was 0.92 (0.81 – 1.05) and the P value was 0.205, confirming that there was no correlation with mortality after LEA. Also, in the univariable logistic regression model, HR (95% CI) was 1.03 (0.92 – 1.16) and P value was 0.609, confirming that there was no correlation with LOS after LEA.

The relationship between HbA1c and the prognosis of DFUs is unclear. In some studies, high HbA1c levels were associated with increased mortality[19, 22], but in most studies, high HbA1c levels were not associated with increased mortality[23, 24, 25, 26, 27, 28, 29]. Also in our

study, this association is not significant in Cox proportional hazards regression and logistic regression. This may be due to decreased insulin clearance and increased occurrence of CKD in patients who died. However, HbA1c is less reliable in patients with CKD and its association with death may be related to increased medical management, hospitalizations, and poor appetite in more unwell patients[24].

In another meta-analysis[30], HbA1c was not found to be a significant predictor of amputation in DFUs as there was no significant difference in the baseline HbA1c levels between patients with LEA and those without. The recent intensive treatment for DFUs may have offset the pre-existing poor diabetic control reflected in HbA1c levels, but the role of glycemic control in the prognosis of DFUs requires further investigation in prospective studies.

Based on the findings from our study, it was observed that major amputees did not show a statistically significant difference in terms of mortality and LOS when compared to minor amputees in patients with DFUs. In the univariable Cox proportional hazard regression analysis of LEA patients, the HR was 1.58 (95% [CI]: 0.84 – 2.99) with a p-value of 0.156, indicating no significant correlation with mortality after LEA. Similarly, in the univariable logistic regression model, the HR was 1.61 (95% CI: 0.82 – 3.18) with a p-value of 0.170, confirming that there was no significant correlation with LOS after LEA.

These findings suggest that there may be no significant difference in mortality and LOS between major and minor amputations in patients with DFUs. This conclusion challenges the notion that major amputations are inherently associated with worse outcomes compared to minor amputations. It is important to note that our study took into consideration various factors that could impact outcomes, such as age, disease severity, comorbidities, and other relevant patient characteristics, to minimize confounding effects and provide a robust analysis. These results are consistent with some previous studies[17, 31] that have also reported

no significant difference in survival rate between major and minor amputations in patients with DFUs.

However, it is important to acknowledge that the findings from our study and previous studies should be interpreted with caution due to some limitations because only about 11% of the patients had underwent major amputation in our study.

Also, since diabetic amputation patients are often financially difficult, the longer LOS, the greater the financial burden, and the longer the LOS, the worse the prognosis[32,33]. Perelman J et al. found that socioeconomic status, which is not currently classified as risk adjuster, has a significant effect on LOS. Accordingly, high-income earners, self-employed people, and office workers had shorter LOS, and the opposite was the case for inactive low-income patients and patients with preferential insurance[34]. As such, the LOS and the patient's financial factors are closely related. However, this study was conducted in Korea, where more than 95% of the population is enrolled in the National Health Insurance and has easy access to the medical system, so the impact on the patient's economy could be minimized. In previous studies, ESR, HbA1c, BMI, CVA or old CAD were considered as factors affecting the LOS of patients with DFUs[35], or severity of the wound, WBC, CRP, and albumin were considered[36]. Therefore, this study is the first to consider RDW as a factor influencing the LOS of patients with DFUs. In this study, it was confirmed that there was a statistically significant relationship between RDW levels of 14.5% or more and a LOS of 30 days or more.

Several possible mechanisms can be considered as mechanisms by which the increase in RDW affects the increase in mortality and the LOS

First, short telomere length may be considered a cause of increased RDW affecting mortality and prolonged LOS[37]. Shorter telomere lengths cause a decrease in RBC count, increase in MCV, decrease in hemoglobin, and increase in RDW. Shorter telomere lengths

are well known as a factor related to aging in general[38, 39], but they are also related to various diseases as well as aging. It is also associated with the development of DFUs[40]. Second, an increase in RDW is associated with oxidative stress. Increased oxidative stress and inflammation appear to produce free radicals that damage red blood cells, changing their morphology, which can affect other hemorheological parameters. Changes in erythrocyte morphology can negatively affect small blood vessel circulation and gas exchange[41]. In addition, peroxidase protects erythrocytes from oxidative damage, and in humans, selenium supplementation increases glutathione peroxidase activity in erythrocytes, and serum selenium can inhibit the increase in RDW by protecting erythrocytes from oxidative damage[42]. This suggests that oxidative stress may be a biological mechanism for the increase in RDW[43]. Third, increased RDW may be related to inflammation. Inflammation can impair erythrocyte maturation and allow immature erythrocytes to enter the bloodstream, which can be achieved by promoting anisocytosis through impaired iron metabolism and interruption of the erythropoietin response[44]. Inflammation can also create a condition in which immature and mature erythrocytes are mixed together and the survival rate of erythrocytes can be reduced[45]. It is thought that this will eventually lead to an increase in RDW.

Mortality increase and long-term hospitalization are also possible due to the decrease in the oxygen carrying capacity of red blood cells with increased RDW. In patients with cardiovascular disease, higher RDW levels were associated with decreased peak VO2, suggesting impaired oxygen transport capacity[46]. The reason for this association is not clear, but it may be due to the presence of immature red blood cells with reduced oxygen-binding capacity or the presence of acquired red blood cell injuries such as decreased deformability, decreased hemoglobin content, increased oxygen affinity, and constrained energy metabolism, among others. The RDW changes observed in critically ill patients may reflect

the presence of these blood cell injuries and serve as a biomarker for impaired oxygen delivery capacity[47].

V. CONCLUSION

Age, ESRD, and RDW influence prognosis in diabetic foot amputation patients. Among them, RDW is highly correlated with prognosis, and high RDW over 14.5% value is an independent prognostic factor with increased mortality and prolonged LOS, implying that RDW may be a simple and inexpensive laboratory parameter for risk stratification in diabetic foot amputation.

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ABSTRACT(IN KOREAN)

적혈구 크기 분포 (RDW)의 당뇨병성 족부 절단술 후 예후와 관련된 예측 인자로서의 가치

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조 항 환

적혈구 크기 분포(RDW)는 적혈구 크기의 이질성 정도를 나타낸다. 지난 수십 년간 RDW 와 다양한 질환의 관계에 대한 연구 수는 기하급수적으로 증가하였다. 그러나 RDW 와 당뇨병성 족부 절단술의 관련성은 현재까지 평가되지 않았다. 본 연구에서는 당뇨병성 족부 절단술에서 RDW 의 예후 인자로서의 가치를 평가하였다. 2009 년 1 월부터 2019 년 1 월까지 당뇨병성 족부 절단술을 받은 415 명의 당뇨병성 족부환자의 자료를 후향적으로 분석하였다. 모든 사망원인에 대한 RDW 의 최적 결정점을 설정한 후 생존율을 위한 Cox 비례위험 회귀 모형과 30 일 이상의 입원 기간에 대한 로지스틱 회귀분석을 통해 RDW 수치, RDW 외 혈액검사 결과, 인구통계학적 변수 및 동반질환 등의 중요한 예후 인자를 평가하였다. RDW 결정점(cut-off value) 14.5%는 모든 원인으로 인한 사망률 증가와 유의한 관련이 있는 것으로 나타났다($P < 0.001$). 다변량 조정 회귀 분석에서 높은 RDW 수치는 모든 사망률 증가의 유의한 위험 인자였다(위험비[HR]: 2.42, 95% 신뢰 구간 [CI]: 1.46 - 4.00). 또한 높은

RDW 수치는 더 길어진 입원 기간과 관련이 있었다(오즈 비율: 2.17, 95% CI: 1.29 - 3.66). 따라서 14.5% 이상의 높은 RDW 수치는 사망률 증가와 입원 기간 연장과 연관된 독립적인 예후 인자이며, RDW 는 당뇨병성 족부 절단술에서 위험 분류를 위한 간단하고 경제적인 검사 항목일 수 있다.

핵심되는말 : 당뇨병성 족부, 절단술, 적혈구 크기 분포, 사망률, 입원 기간