Original Article

High Preoperative Neutrophil-Lymphocyte Ratio (NLR) and Red Blood Cell Distribution Width (RDW) as Independent Predictors of Native Arteriovenous Fistula Failure

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Objective: To study the association between a high preoperative neutrophil lymphocyte ratio (NLR) and red cell distribution width (RDW) with arteriovenous fistula (AVF) failure, as well as to determine the cut-off values in a South Asian population.

Materials and Methods: A total of 150 consecutive patients with a failed fistula who presented in the Department of Vascular Surgery between January 2014 and January 2016. Patients fulfilling the inclusion criteria were selected as defined as Case. They were compared with 150 patients who had matured fistulae (Control).

Results: A significant difference was found between the Case and Control groups in mean preoperative NLR (3.3±0.5 versus 2.2±0.9, P value=0.011) and RDW (15.9±2.9 versus 13.6±1.1%, P value of 0.02), respectively. Multivariate analysis revealed that NLR (Odds Ratios (OR) 1.39; 95% Confidence Intervals (CI) 1.02 to 2.08; P<0.001) and RDW (OR 1.39; 95%CI 1.11 to 1.69; P<0.001) were strong independent predictors of AVF failure. A receiver operating characteristic curve analysis showed a cut-off value of 2.65 (specificity 80%, sensitivity 98%) and 15.1 (specificity 79%, sensitivity 98%) for NLR and RDW, respectively.

Conclusion: Increased preoperative NLR and RDW were associated with a high rate of AVF failure in a South Asian population.

Keywords: arteriovenous fistula, neutrophil lymphocyte ratio, red cell distribution width

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Introduction

The most common cause of access failure is stenosis, and the development of thrombosis in the immediate post-stenotic site.[1] The stenosis results from neointimal hyperplasia and a vast array of inflammatory factors.[1–4] Various inflammatory predictive parameters to assess association of arteriovenous fistula (AVF) failure and inflammation have been studied, such as neutrophil lymphocyte ratio (NLR), red cell distribution width (RDW), white blood cells (WBC) count, Interlukin-6, C-reactive protein (CRP), and erythrocyte sedimentation rate.[3–5]

NLR is a very simple indicator to assess systemic inflammation. NLR has been used as an indicator to assess coronary stenosis and to predict survival in patients with acute coronary syndrome.[6] Furthermore, it is a strong predictor in stent failure due to restenosis.[7] Studies have shown an association of high preoperative NLR with failure of a saphenous vein graft due to stenosis in coronary bypass grafting.[8] Since NLR is a strong predictor of stenosis secondary to inflammation, and inflammation plays an important role in AVF stenosis and failure, the association of NLR with AVF stenosis is an area of interest.

RDW is another parameter, which has strong association with inflammation and subsequently AVF failure.[9] Studies claim RDW to be a better marker than CRP and WBC count.[10]

Considering the ease and availability of RDW and NLR in a simple blood test, this prospective cohort study was designed to test whether raised NLR and RDW are independent predictors of AVF failure. Additionally, what are the likely preoperative cut-off values that can be clinically useful to predict AVF failure in the South Asian population.

Patients and Methods

All patients reporting to Vascular Surgery Clinic in Com-

bined Military Hospital Lahore between January 2014 and January 2016 for AVF creation, who fulfilled the inclusion criteria, were included in this study. All patients underwent a Duplex Scan with 5 MHz linear probe by a consultant radiologist to assess the superficial veins of the upper limb for thrombosis, stenosis, or aneurysmal dilation. All such patients were excluded from the study. Patients who had previous surgery on the arm, advanced cardiac failure [New York Heart Association (NYHA) functional classification stage 3 or 4], hematological malignancy, bleeding disorder, or using anticoagulants were also excluded from the study.

Those who fulfilled the inclusion criteria had their blood samples taken on the day of the surgery prior to the procedure. Blood samples were tested for the parameters described in **Table 1**. All patients underwent an AVF procedure by a consultant vascular surgeon, who had a minimum of five years of experience of doing such procedures

under local anesthesia. Patients were given oral antibiotics and analgesia on discharge. They were provided written instructions about hand and forearm exercises and general care of the AVF.

Patients were regularly followed up in the clinic at 2 weeks, 4 weeks, 2 months, and then every 3 months for a minimum of one year. A functionally mature AVF is defined by Kidney Disease Outcome Quality Initiative (KDOQI) guidelines as one that can be easily cannulated and has at least six successful consecutive dialysis sessions.[11] The primary outcome of the study was failure of the fistula.

The data was analyzed using the Statistical Package for Social Sciences version 20.0. The numerical data, such as age was calculated as the mean and standard deviation. Categorical data, such as gender was recorded as frequency and percentage and compared between the groups using a Chi-Square test. Patients were divided into two

Table 1 Clinical and biochemical parameters of the patients

Patient parameters	Fistula matured (Group A)	Fistula failed (Group B)	P value	All patients
Total patients (N)	150	150		300
Mean age (Years±SD)	45±13	43±15	0.847	44±12
Gender male [n, (%)]	118 (51.3)	112 (48.7)	0.792	230 (76.6)
Gender female [n, (%)]	32 (45.7)	38 (54.2)	0.527	70 (23.3)
Ejection fraction (%±SD)	59±5	58±6	0.691	
NYHA class 1 (n)	101	97	0.449	198
NYHA class 2 (n)	49	53	0.521	102
Atherosclerotic risk factors:				
Smoker [n, (%)]	84 (56)	79 (52.6)	0.679	163 (54.3)
Ischemic heart disease [n, (%)]	24 (16)	27 (18)	0.592	51 (17.0)
Systolic BP (mmHg±SD)	142±13	143±12	0.773	
Diastolic BP (mmHg±SD)	93±9	94±11	0.431	
Diabetes mellitus [n, (%)]	61 (40.6)	52 (34.6)	0.511	113 (37.6)
Biochemical parameters:				
HDL cholesterol (mg/dL±SD)	49.4±11.9	50.2±12.2	0.629	
LDL cholesterol (mg/dL±SD)	101.4±32.8	100.7±37.2	0.482	
Triglycerides (mg/dL±SD)	192.2±83.4	195.5±91.1	0.745	
HbA$_1$c in diabetic patients (%±SD)	7.9±0.8	8.1±0.9	0.091	
White cell count (10^9/L±SD)	7.4±2.1	8.1±1.9	**0.024**	7.9±1.8
Neutrophils (10^9/L±SD)	4.8±1.7	5.6±1.4	0.068	4.9±2.1
Lymphocytes (10^9/L±SD)	1.7±0.2	1.8±0.4	0.215	1.7 ±0.5
Monocytes (10^9/L±SD)	0.5±0.1	0.7±0.4	0.414	0.6±0.1
NLR (×± SD)	2.2±0.9	3.3±0.5	**0.011**	2.6±0.7
Hematocrit (%±SD)	29.4±3.4	33.2±2.9	0.082	31.2±1.9
RBCs (×10^{12}/L±SD)	3.1±0.8	3.6±0.5	**0.049**	3.4±0.6
Hemoglobin (g/dL±SD)	9.2±1.1	10.3±1.5	0.075	9.8±1.1
MCV (fL±SD)	88.4±8.9	89.9±7.4	0.871	89.2±6.9
MCH (pg±SD)	30.1±2.9	30.6±3.1	0.894	30.4±2.6
RDW (%±SD)	13.6±1.1	15.9±2.9	**0.020**	14.4±1.9
Platelets (×10^3/μL±SD)	229±75	258±79	0.061	248±81

groups. Group A was defined as patients with a mature fistula, and Group B was defined as a failed fistula. The comparison between the two groups was performed using a Student's t test. The association of laboratory markers with the maturation (yes) or failure (no) of AVF was analyzed using univariate logistic regression. Stepwise reduction was performed using multivariate regression for those variables when the P value in univariate regression was significant. A receiver operating characteristic (ROC) curve analysis was done to establish the cut-off value for all of the statistically significant variables of multivariate regression. Odds Ratios (OR) and 95% Confidence Intervals (CI) were calculated. The results were considered statistically significant if the P value was found to be less than or equal to 0.05.

Results

A total of 365 consecutive patients fulfilling the inclusion criteria were included in the study. Of these, 65 were excluded (39 died and 26 lost to 1 year follow up). There were 150 patients with a failed fistula. Of the 150 patients with a failed AVF, 31 (20.7%) had early failure (defined as failure to mature within the first 8 weeks) and 119 (79.3%) had late failure. The average time to failure in this group was 5 ± 4 months. Patients with a failed fistula were assigned to Group B (Case). Group A comprising of similar number of consecutive patients who had their fistula matured were selected for comparison (Control).

The minimum age of patients was 21, and the maximum age was 62 years with mean age of 44 ± 12 years. Out of these 300 cases, 230 (76.6%) were males and 70 (23.3%) were females. The male to female ratio was $3.2:1$. There

was no difference in age and gender between the two groups. Similarly, major risk factors for atherosclerotic disease, such as diabetes, hypertension, and smoking were not statistically significant different between the two groups. Furthermore, there were no differences in cardiac status when compared in terms of ejection fraction (59 ± 5 mature versus 58 ± 6 failed group; $P = 0.691$) and NYHA class (**Table 1**).

Patients with a failed fistula had a higher WBC count when compared to the patients with matured fistulae (8.1 ± 1.9 versus $7.4 \pm 2.1 \times 10^9$/L; $P = 0.024$). Similarly, they had higher hematocrit (33.2 ± 2.9 versus $29.4 \pm 3.4\%$; $P = 0.082$), red blood cells (RBCs) count (3.6 ± 0.5 versus $3.1 \pm 0.8 \times 10^{12}$/L; $P = 0.049$), and hemoglobin (10.3 ± 1.5 versus 9.2 ± 1.1 g/dL; $P = 0.075$). The mean NLR levels in patients with failed fistula group were higher in comparison to the matured fistula group (3.3 ± 0.5 versus 2.2 ± 0.9) with a P value of 0.011, which is statistically significant. Furthermore, when RDW was compared, it also showed a statistically significant difference between the two groups (15.9 ± 2.9 in fistula failure group versus $13.6 \pm 1.1\%$ in fistula mature group) with a P value of 0.02. No significant differences were found in the remainder of the biochemical parameters analyzed.

On further analysis of the failed group, the mean NLR was higher in patients who had early failure of fistula when compared to late failure group (3.5 ± 0.5 versus 3.1 ± 0.4; $P = 0.049$). The difference was statistically significant. For RDW, no significant difference was found between the two failure groups (15.7 ± 3.1 early versus 16.0 ± 2.6 late failure; $P = 0.054$). No significant difference was found between the failed sub-groups in the other biochemical parameters described in **Table 1**.

Table 2 Outcome of the univariate and multivariate analysis

Biochemical parameters	Univariate analysis ORs (95%CI)	P value	Multivariate analysis ORs (95%CI)	P value
HDL cholesterol (mg/dL±SD)	0.98 (0.94–1.1)	0.351		
LDL cholesterol (mg/dL±SD)	0.99 (0.97–1.1)	0.561		
Triglycerides (mg/dL±SD)	1.00 (1.00–1.1)	0.766		
White cell count (10⁹/L±SD)	1.16 (1.02–1.45)	0.044		
Neutrophils (10⁹/L±SD)	1.22 (1.00–1.69)	0.061		
Lymphocytes (10⁹/L±SD)	1.25 (0.97–1.85)	0.598		
Monocytes (10⁹/L±SD)	0.65 (0.05–1.21)	0.368		
NLR (×±SD)	1.12 (0.95–1.54)	0.011	1.39 (1.02–2.08)	<0.001
Hematocrit (%±SD)	0.88 (0.76–0.99)	0.063		
RBCs (×10¹²/L±SD)	0.51 (0.32–0.98)	0.039	0.96 (0.20–3.69)	0.893
Hemoglobin (g/dL±SD)	0.80 (0.65–1.02)	0.075		
MCV (fL±SD)	0.99 (0.89–1.05)	0.726		
MCH (pg±SD)	0.92 (0.77–0.99)	0.594		
RDW (%±SD)	1.05 (0.99–1.41)	0.011	1.39 (1.11–1.69)	<0.001
Platelets (×10³/μL±SD)	0.99 (0.91–1.03)	0.742		

The results of univariate analysis show that an increase in WBC count (OR 1.16; 95%CI 1.02 to 1.45; P = 0.044), NLR (OR 1.12; 95%CI 0.95 to 1.54; P = 0.011), RBC count (OR 0.51; 95%CI 0.32 to 0.98; P = 0.039) and RDW (OR 1.05; 95%CI 0.99 to 1.41; P = 0.011) significantly increase the risk of fistula failure (**Table 2**). Since hemoglobin and hematocrit are strongly correlated with RBCs count (r^2 = 0.81 and 0.85 respectively, both with a P value of < 0.001), they were excluded from multivariate analysis. Neutrophils and WBCs count were significantly correlated with NLR (r^2 = 0.82 and 0.83 respectively; P < 0.001), so they were also excluded. Multivariate analysis revealed that NLR (OR 1.39; 95%CI 1.02 to 2.08; P < 0.001) and RDW (OR 1.39; 95%CI 1.11 to 1.69; P < 0.001) are strong independent predictors of AVF failure.

ROC curve analysis was done to determine the cut-off values for NLR and RDW. The optimal cut-off value for NLR was 2.65. The area under curve (AUC) was 0.792 (0.711–0.843) with a specificity of 80% and sensitivity of
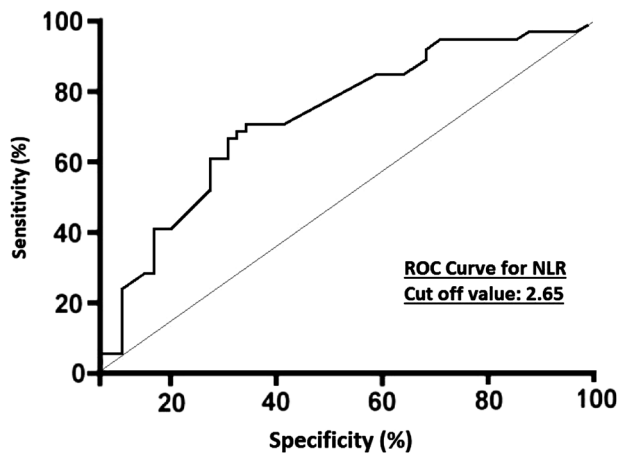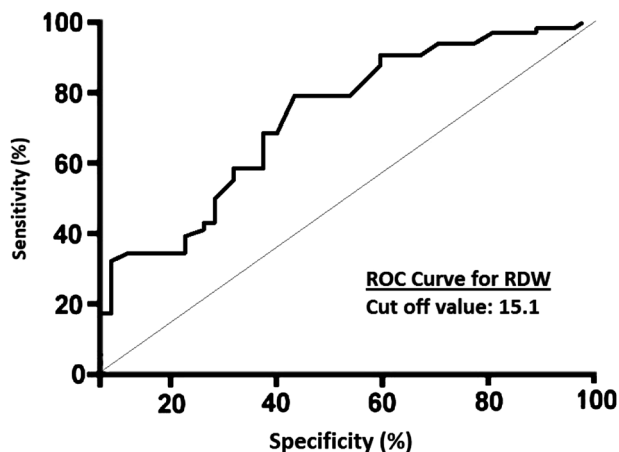

Fig. 1　ROC curve analysis for NLR.

Fig. 2　ROC curve analysis for RDW.

98% (**Fig. 1**). **Figure 2** shows the cut-off value for RDW as 15.1, with a specificity of 79%, sensitivity of 98% and AUC as 0.821 (0.764–0.901).

Discussion

Studies suggest that inflammation causes neointimal hyperplasia, which results in thrombosis at the anastomotic site and is responsible for failure of the AVF.[4,12,13] Neointimal hyperplasia is pathologically similar to atherosclerosis.[14] Studies have clearly demonstrated that atherosclerosis is the main pathology in coronary occlusive disease,[6] bare metal stent failure[7] and autologous saphenous vein graft failure[8]; and NLR has been shown as a strong independent predictor in all of these scenarios. Considering atherosclerosis as the primary mechanism responsible for AVF stenosis and failure, and NLR being a strong predictor of atherosclerosis elsewhere; it can also be a positive predictor of AVF failure. In our study, we have revealed a clear association between inflammation and AVF failure using NLR. The results are consistent with other studies showing similar association between NLR and inflammation, leading to AVF failure.[2–4]

In our study, we have clearly demonstrated using multivariate logistic regression that raised preoperative NLR is a strong independent predictor of AVF failure (OR 1.39; 95%CI 1.02–2.08; P < 0.001). Similar results are advocated by Yilmaz et al. in his logistic regression model of 108 patients (OR 4.734; 95%CI 2.987–7.321; P = 0.001).[15] Yilmaz et al. concluded that a raised NLR is a strong predictor of AVF failure, and its correlation with failure is due to inflammation and atherosclerosis resulting in neointimal hyperplasia.[15]

The mean normal value for NLR is variable depending upon age and ethnicity.[16] Its reported values in the African population is 2.8, while it is much lower in the American white population (1.76).[16,17] It was even lower in healthy Chinese population (1.5).[18] Studies also suggest that the mean NLR is lower in a healthy age group of 18–50 years when compared with 51–80 years.[16] Considering this variation, we performed this study determine the cut-off values of NLR in our South Asian population. Various studies have concluded that cut-off values for NLR could be useful in predicting the AVF failure. Çildağ et al. proposed a cut-off value of NLR as 3.18 for the determination of primary patency (sensitivity 81.4%, specificity 51.4%).[19] Yilmaz et al. concluded a cut-off value of 2.70 for AVF stenosis (sensitivity 98.4%, specificity 75%).[15] In our study, we also calculated the cut-off value using the ROC curve analysis. The value was 2.65 with a specificity of 80% and sensitivity of 98%.

RDW is another simple blood parameter, which is routinely reported in automated laboratory blood count

equipment. RDW is calculated as standard deviation of red blood cell volume divided by the mean corpuscular volume (MCV). RDW has been reported as a strong independent predictor of mortality in patients with coronary occlusive disease. The exact mechanism is unknown; however, it is suggested that high RDW is an indicator of poor red cell production, malnutrition, and inflammation.[9] One can argue that a raised RDW can be due to anemia reflected by low hematocrit, hemoglobin, and RBCs levels, suggesting that confounding factors in the results. Univariate analysis of the two variables (hemoglobin and hematocrit) did not show an association between them and the raised RDW, since the P values were not significant in either case. RBCs levels were subjected to multivariate analysis, and the P value failed to show any statistically significant association between RBCs and RDW. We concluded that the increased RDW is an independent predictor for AVF failure. Similar results were proposed by Bojakowski et al. in 2012, when they concluded that raised RDW is a better predictor of AVF failure than other inflammatory markers such as raised WBC count and CRP, suggesting RDW as an independent predictor of AVF failure.[10]

The strength of this study is its prospective design, non-purposive consecutive sampling, standardized preoperative biochemical blood parameter measurements, univariate and multivariate analysis of parameters, and proposed cut-off values of statistically significant parameters for a South Asian population. However, this is a single center study with no randomization of the study groups. Other factors, which may affect maturation of fistula, such as small vein caliber, obesity, low flow rate, anatomical location of access site in terms of depth and tortuosity, non-standardization of the fistula access protocols in terms of experience of dialysis staff, and patient education could be potential confounding factors affecting the final results. We propose larger multicenter randomized trials with stricter preoperative vascular assessment and standardized protocols for dialysis access to keenly assess the association between these simple markers and the fistula failure rate.

Conclusion

Raised preoperative NLR and RDW were found to be associated with a high rate of AVF failure in a South Asian population.

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All authors have no conflict of interest.

Author Contributions

Study conception: RU
Data collection: RU, MN
Analysis: RU, MJ
Investigation: RU, MN
Writing: RU, MJ
Critical review and revision: RU, MN
Final approval of the article: all authors
Accountability for all aspects of the work: all authors

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