Red Cell Distribution Width and Neutrophil/lymphocyte Ratio in Bare Metal Coronary Artery Stent Restenosis

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Abstract  Background: Coronary stents are commonly used to treat obstructive coronary artery disease. It is currently difficult to reliably predict in-stent restenosis. The aim of this study was to examine the relationship between bare metal stent restenosis and red cell distribution width (RDW), neutrophil/lymphocyte ratio (N/L ratio). It was the first study that used a control group with a normal coronary angiogram.

Methods: We enrolled patients who underwent coronary angiography between June 2012 and September 2013 in our center. We enrolled a cohort of 210 consecutive patients, of which 130 had a coronary artery stent and 80 had a normal coronary angiogram.

Results: The mean age of the study group was 62 (38-86) years. The mean RDW levels of patient group with no-restenosis were significantly higher than the control group but there was no significant difference between the mean RDW levels of the restenosis group and the other subgroups (14.9 (11.7-17.6), 15.5 (10.9-20.4), 15.4 (11.7-20.1), respectively). N/L ratio was significantly higher in patients with stent restenosis 2.32 (1.49-5.35) compared to the other two groups whereas there was no significant difference between the control group and non-restenosis group with respect to N/L ratio (1.71(0.84-7.89), and 2.09(0.89-9.15), respectively).

Conclusions: According to our findings, RDW was not a predictor of stent restenosis or coronary artery disease. On the other hand, our findings support the hypothesis that N/L ratio is an indicator of inflammation that plays a role in-stent restenosis.

Keywords: Coronary restenosis; Lymphocytes; Neutrophils; Red cell distribution width; Stents

1. Introduction

Coronary stents are commonly used to treat occlusive coronary artery disease. Despite promising improvements in stent technology, the optimum treatment strategy for patients requiring coronary artery stenting is still not fully understood, due particularly to the difficulty to predict which patients will develop in-stent restenosis (ISR). Neointimal hyperplasia of matrix and smooth muscle cells is a major cause of ISR [1]. Stent implantation causes injury to the coronary vessel endothelium, initiating both local and systemic inflammatory responses, which play an important role in the pathophysiology of ISR [2,3]. Several studies have investigated the predictive role of laboratory parameters, measured by complete blood count or biochemical analysis before the coronary stenting procedure in ISR. Red blood cell distribution width (RDW), which is reported routinely in complete blood count analyses, is a measure of the variability of the size of circulating erythrocytes [4]. Increased RDW has been shown to be associated with adverse clinical outcomes in patients
with heart failure, ST-elevation myocardial infarction, and stable angina pectoris (SAP) [5]. However, the mechanism underlying increased RDW level and adverse cardiovascular outcomes has been poorly understood. Inflammation may disturb red cell membrane leading to changes in red blood cell maturation, resulting in an increased RDW [6]. As with RDW, relative ratios of white blood cell (WBC) subtypes, especially the neutrophil-to-lymphocyte ratio (NLR), have been proposed as a prognostic marker and related to a proinflammatory state imposing worse cardiovascular outcomes in cardiovascular disease [7-9].

The aim of this study was to examine the relationship between red cell distribution width, neutrophil/lymphocyte ratio and bare metal stent restenosis. Although there have been studies examining these markers in the literature, this is the first study that used a control group with a normal coronary angiogram.

2. Materials and Methods

We enrolled patients who underwent coronary angiography between June 2012 and September 2013 in our center. We also enrolled a control group of patients who met criteria for undergoing coronary angiography at their first examination and found to have a normal coronary angiogram.

Coronary angiographic examination was performed under local anesthesia via the femoral artery. All coronary arteries were visualized in the right and left anterior oblique projections with caudal and cranial angulations, and in the left lateral projection (Philips Artis zee, Munich, Germany). Iohexol was used as the contrast agent. Coronary angiography views were assessed by two experienced cardiologists who had no knowledge of the patients’ clinical or demographic characteristics. The extent of coronary stenosis was determined at the projection which showed the stenosis at its most severe form. Quantitative coronary angiography was used to determine the severity of stenosis, with angiographic restenosis having been defined as ≥50% luminal narrowing [10].

For all patients, a preoperative complete blood cell count, white blood cell subgroup analysis, fasting blood glucose, urea, creatinine, high density lipoprotein, triglyceride, low-density lipoprotein, aspartate aminotransferase, and alanine aminotransferase levels were recorded.

We excluded patients who had acute coronary syndrome; advanced cardiac failure; severe cardiac valve dysfunction; chronic renal disease represented by a serum creatinine concentration >2 mg/dl; active infection; chronic inflammatory disease and cancer.

Written informed consent was obtained from all participants and the protocols approved by the Ethics Committee were utilized throughout the study. A standardized questionnaire was used to collect clinical and demographic information, including medication history.

Heart failure was defined as a clinical diagnosis according to the patient history and the use of drugs. Previous atrial fibrillation (AF) was defined as a history of any AF or presence of AF on reference electrocardiogram performed in outpatient clinic. ECG changes was defined as horizontal or down-sloping ST depression ≥0.05 mV (0.5 mm) in two anatomically contiguous leads or T inversion ≥0.1 mV (1 mm) in two anatomically contiguous leads with prominent R wave or R/S ratio >1 or presence of pathological Q waves. Contiguous leads are defined as pairs or groups of leads that reflect the different walls of the heart. These are the inferior (II, III, aVF), lateral (I, aVL), and anterior leads (V1 to V6).

Patients were divided into three groups on the basis of their angiographic characteristics: those with in-stent restenosis, those without in-stent restenosis and the control group with a normal coronary angiogram. Red cell distribution width (RDW), neutrophil/lymphocyte ratio (N/L ratio) and other recorded variables were compared between the three groups using Kruskall Wallis test as a postHoc analyses paired Mann Whitney-U test was utilized to identity significantly different pairs. Categorical data were analyzed with Fisher’s exact test and the Chi-squared test. Normally distributed continuous variables were expressed as the mean ± standard deviation, non normally distributed continous variables were expressed as median (min-max); categorical variables were presented as number (%). Backward stepwise logistic regression analyses method was used for multivariate analyses. The significant covariables were put in multivariate analyses. A P-value <0.05 was considered statistically significant. All analyses were performed using the SPSS statistical software package (version 17; SPPS, Chicago, IL, USA).
3. Results

We enrolled a cohort of 210 consecutive patients undergoing coronary angiography, of which 130 patients had bare metal coronary artery stents and 80 had normal coronary angiograms (the control group). Of those with a coronary artery stent, 50 had in-stent restenosis whereas 80 did not. The mean age of the overall study population was 62 (38-86) years, and 120 (57.1%) of them were male. The mean time from stent implantation to the surveillance angiography was 36.7±35.1 months. A comparison of the demographic and clinical characteristics of the groups was shown on Table 1.

Serum lipid concentrations were significantly lower in the study group than the control group, an effect that was attributable to statin use. According to drug use of patients there were no significant difference between no restenosis group and in stent restenosis group. It was an expected result that control group was significantly different according to usage of statin, asetyl salisilic asid, clopidogrel, nitrates, beta-blocker and ACEI/ARB therapy. The mean RDW levels of patient group with no-restenosis were significantly higher than that of the control group but there was no significant difference between the mean RDW levels of the restenosis group and the other subgroups (14.9 (11.7-17.6), 15.5 (10.9-20.4), 15.4 (11.7-20.1)respectively). N/L ratio was significantly higher in patients with stent restenosis 2.32(1.49-5.35) compared to the other two groups whereas there was no significant difference between the control group and non-restenosis group with respect to N/L ratio 1.71(0.84-7.89), and 2.09(0.89-9.15) respectively). A comparison of the laboratory parameteres were shown on Table 2.

In univariate analyses ischemia findings in electrocardiography, N/L ratio and previous myocardial infarction history was found statistically significant. According these covariables, in multivariate analysis we found ischemia findings in electrocardiography was the main predictor. It increases the risk for instent restenosis between 2.7 to 14.4 times ($x^2=6.32$). But N/L ratio was not a independent predictor for stent restenosis ($p=0.387$) (Table 3). In ROC curve analyses for N/L ratio, it is shown that at a cut-off point of 1.845, NLR can distinguish stent restenosis with a high sensitivity (90%) and a moderate specificity (52.5%) (Figure 1).

Table 1. Comparison of the demographic and clinical characteristics

|                | Control Group | Stent restenosis (-) | Stent restenosis (+) | p value |
|----------------|---------------|-----------------------|----------------------|---------|
|                | n=80          | n=80                  | n=50                 |         |
| Age, years     | 61.5 (38-79)  | 63 (38-83)            | 62 (38-86)           | 0.250   |
| Male sex, %    | 46.3          | 61.3 ^a               | 68 ^a                | 0.033   |
| Hypertension, %| 62.5          | 81.3 ^a               | 70                   | 0.031   |
| Diabetes mellitus, % | 26.5          | 42.5                  | 34                   | 0.097   |
| Hyperlipidemia, % | 57.5          | 88.6 ^a               | 84 ^a                | 0.001   |
| History of smoking, % | 35            | 26.3                  | 34                   | 0.446   |
| History of familial CAD, % | 40            | 32.5                  | 36                   | 0.615   |
| CABG, %        | 0             | 8.8 ^a                | 10 ^a                | 0.019   |
| Previous MI, % | 0             | 37.5 ^a               | 64 ^a ^b             | 0.001   |
| Atrial fibrillation, % | 6.3           | 6.3                   | 6                    | 0.998   |
| Heart Failure, % | 3.8           | 16.3 ^a               | 20 ^a                | 0.010   |
| Ischemia on ECG, % | 18.8          | 38.8 ^a               | 80 ^b ^a             | 0.001   |
| Wall motion abnormalities in Echocardiography, % | 20           | 35                    | 50 ^a                | 0.003   |

Abbreviations: CABG, coronary artery bypass grafting; CAD, coronary artery disease; ECG, electrocardiography; MI, myocardial infarction.

^a: versus control group (Chi-squared test)

^b: Versus the non- restenosis group (Chi-squared test)
Table 2. Comparison of the laboratory investigations

|                     | Control Group n=80 | Stent restenosis (-) n=80 | Stent restenosis (+) n=50 | p value |
|---------------------|--------------------|---------------------------|---------------------------|---------|
| Fasting blood glucose, mg/dl | 101 (83-199) | 110.5(62-291) | 105(86-277) | 0.06 |
| Creatine, mg/dl | 0.76 (0.60-1.51) | 0.83(0.38-1.59) *a | 0.90(0.59-3.10) *a | 0.003 |
| HDL-C, mg/dl | 42(23-75) | 38(17-60) *a | 38(24-72) | 0.003 |
| LDL-C, mg/dl | 125.5(55-221) | 100(37-183) *a | 98.5(43-202) *a | 0.001 |
| TG, mg/dl | 133.5(60-871) | 133.5(56-589) | 133.5(48-481) | 0.996 |
| Total cholesterol, mg/dl | 198(118-280) | 169.5(95-265) *a | 172.5(115-337) *a | 0.001 |
| ALT, U/L | 18(6-73) | 19(7-60) | 18(8-45) | 0.254 |
| Hemoglobin, g/dl | 14(10.8-16.5) | 14.2(9.6-17.0) | 13.8(9.4-17.7) | 0.598 |
| White Blood Cell, 10^3/uL | 6.90(3.6-13.9) | 7.8(4.1-12.4) | 7.65(4.7-12.1) | 0.140 |
| Platelets, 10^9/uL | 260.5(161-413) | 245(114-426) | 242.5(118-465) | 0.198 |
| RDW | 14.9(11.7-17.6) | 15.5(10.9-20.4) *a | 15.4(11.7-20.1) | 0.002 |
| Neutrophil count, x10^9/L | 4.01(1.57-9.07) | 4.65(2.10-8.90) *a | 5.06(2.65-10.70) *a | 0.001 |
| Lymphocyte count, x10^9/L | 2.24(1.06-4.58) | 2.29(0.84-4.5) | 2.03(1.01-4.30) | 0.067 |
| N/L ratio | 1.71(0.84-7.89) | 2.09(0.89-9.15) | 2.32(1.49-5.35) *a,b | 0.001 |

Abbreviations: ALT, alanine aminotransferase; HDL-C, high density lipoprotein; LDL-C, low density lipoprotein; N/L ratio, neutrophil/lymphocyte ratio; RDW, red cell distribution width; TG, triglyceride.

a : vs control group (Mann-Whitney-U test)
b: Versus the non- restenosis group (Chi-squared test)

Table 3. Multivariate analysis of risk factors for stent restenosis

| Variable            | p value | x^2   | %95 CI for x^2 |
|---------------------|---------|-------|----------------|
| Previous MI          | 0.634   | 1.24  | 0.50-3.11      |
| N/L ratio            | 0.387   | 1.15  | 0.83-1.58      |
| Ischemia on ECG      | 0.001   | 6.32  | 2.76-14.4      |

Abbreviations: MI, myocardial infarction; N/L ratio, neutrophil/lymphocyte ratio; ECG, electrocardiography
4. Discussion

Our findings showed that there was no significant difference between the restenosis group and no restenosis group with respect to RDW level. RDW level was significantly different between the patients with stent implantation with no-restenosis and the control group. However N/L ratio was significantly different between the restenosis group and the no-restenosis group. It was also different between the restenosis group and the control group. In the literature, a number of studies have investigated RDW and N/L ratio in stent restenosis. Zhao and colleagues [11] compared 45 patients with drug eluting stent restenosis and stable angina pectoris with 247 patients free of restenosis and found a significant relationship between RDW and in-stent restenosis. They also showed that the in-stent restenosis group had a longer stent length and lower stent diameter. Kurtul and colleagues [12] compared 128 patients with stable coronary artery disease and in-stent restenosis and 123 patients without restenosis and found that RDW levels were independently associated with bare-metal in-stent restenosis. In contrast, Tanindi et al [13] found no significant relationship between stent restenosis and RDW values in 285 patients. None of the above mentioned studies recruited patients with normal coronary arteries as the control group. In our study we compared patients with stent restenosis, patients without stent restenosis and patients with normal coronary arteries. We found no significant difference between the restenosis group and no-restenosis group similar to what Tanindi et al [13] reported. However, we found a significant difference between the no-restenosis patient group and the control group with regard to RDW. Therefore, the latter may not be a marker for restenosis and coronary artery disease.

We found a significant different N/L ratio in the restenosis group compared the other two groups. Bolca and colleagues [14] examined a total of 404 patients with bare metal stents (207 patients with stent restenosis and 197 patients without stent restenosis) who were treated for ST segment elevation myocardial infarction. Patients were divided into three groups based on N/L ratio tertiles at hospital admission. They showed a higher N/L ratio in patients with stent restenosis, and that NLR was an independent predictor of stent restenosis. In another study, Turak et al [15] retrospectively analyzed clinical, hematologic, and angiographic data of 624 patients who underwent coronary stent implantation and a subsequent control coronary angiography for stable or unstable angina pectoris. They divided N/L ratio as the previous study. They found the highest N/L ratio in the third tertile, and the N/L ratio was an independent predictor of bare metal restenosis. Balli and colleagues [16] investigated the relationship between N/L ratio and stent restenosis in bifurcation lesions in a cohort of 181 stable coronary artery patients who had undergone stent implantation to bifurcation lesions. Higher levels of N/L ratio were detected in patients with in-stent restenosis. In our study, N/L ratio was studied only once, that is before the control coronary angiography, so we could not evaluate the evolution of N/L ratio prior to and after the procedure. And also there is a relationship between N/L ratio and coronary calcification burden and severity. Ates et al [17] showed that N/L ratio is associated with both severity and morphology of coronary artery disease. Li et al [18] assessed the association between N/L ratio and chronic coronary total occlusion (CTO). They found a significantly higher total white blood cell count,
neutrophil counts, and NLR in the CTO group. In that study the authors measured admission and post-PCI N/L ratios with NLR_A having been defined as the change between those 2 values. In the NLR_A ≥ 0.5 subgroup, the incidence of severe dissection, slow coronary flow, in-stent restenosis (ISR), and major adverse cardiac events was significantly higher. Verdola and colleagues [19] investigated the role of N/L ratio on periprocedural myocardial infarction (MI) in patients undergoing non-urgent PCI in a cohort of 1542 patients undergoing PCI. Higher N/L ratios was related to age, established risk factors and a positive cardiovascular history. NLR was associated with a more severe coronary artery disease, more severe stenosis, more diffuse coronary calcifications, a greater rate of intracoronary thrombus or thrombectomy use. NLR was not predictive of the occurrence of periprocedural MI although it was associated with a higher occurrence of the latter. Our findings suggest that a NLR value exceeding 1.845 can be of use for detecting stent restenosis with a high sensitivity and a moderate specificity. That is, values above this cut-off level can be used to perform further tests to detect stent restenosis in appropriate clinical setting and suggestive symptoms of ischemia. However, it should be noted that, as a marker of inflammation, it lacks a high degree of specificity, and thus should be confirmed by further tests.

5. Study Limitations

Our study has some limitations. The sample size was relatively small, and consequently there may have been too few patients in the in-stent restenosis group. Furthermore, some stents were implanted in other centers; as such, some clinical and demographic data were missing. Consequently, we lack the clinical outcome data and therefore cannot draw any conclusions about predictive power of the RDW and N/L ratio in the occurrence of major cardiovascular events. Future prospective studies with larger patient cohorts are needed to further clarify the relationship between N/L ratio and RDW values and outcomes in patients with coronary artery disease.

6. Conclusion

According to our findings, RDW was not a predictor of stent restenosis or coronary artery disease. On the other hand, although it was not an independent risk factor for stent restenosis, our findings support the hypothesis that N/L ratio is a potential marker of inflammation that plays a role in-stent restenosis.

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