An eBook on Type 2 Diabetes

Chapter 6

Neutrophil - Lymphocyte Ratio, as a Novel Systematic Biomarker: Predicting Intrac-eRebral Hemorrhage in Type 2 Diabetes Mellitus

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Abstract

Introduction: Chronic systematic inflammation has been suggested to be associated with the occurrence and development of cardiovascular events. Low-grade systematic inflammation persists in Type 2 Diabetes Mellitus(T2DM) patients. In addition, the risk of cerebral hemorrhage in these patients is increased compared with non-diabetic patients. Neutrophil-to-Lymphocyte Ratio (NLR) is the ratio derived by dividing the neutrophil count with the lymphocyte count from a peripheral blood sample. This study aimed to explore the relation between NLR and cerebral hemorrhage and to prove that the NLR is an independent risk factor of cerebral hemorrhage in T2DM patients.

Method: In total, 429 cases of T2DM patients were included. The patients were divided into two groups depending on the presence of cerebral hemorrhage: the cerebral hemorrhage group (n=87) and the control group (n=342). Based upon clinical and laboratory data of diabetes diagnosis, this article investigates the relationship between the NLR and risk of cerebral hemorrhage.

Results: The increase in the NLR was positively correlated with the incidence of cerebral hemorrhage in T2DM patients and might serve as an independent risk factor of cerebral hemorrhage in T2DM patients (OR 4.451 95% CI 2.582-7.672). NLR>2.58 might be useful in predicting the threshold value of cerebral hemorrhage risk in newly diagnosed T2DM patients (area under the curve 0.72, 95% CI 0.659-0.780, P<0.001)

Conclusion: As an indicator of the degree of systematic inflammation, NLR is an independent risk factor of cerebral hemorrhage in T2DM patients.

Key words: Intracerebral Hemorrhage; Type 2 Diabetes Mellitus; Neutrophil-to-lymphocyte ratio; inflammation

Abbreviation: T2DM: type 2 diabetes mellitus; ICH: Intracerebral hemorrhage; NLR: neutrophil to lymphocyte ratio; BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein; HbA1c: hemoglobin A1c; FPG: fasting plasma glucose; FIN: fasting plasma insulin; IL-6: interleukin-6; MCP-1: monocyte chemotactic protein-1; CRP: C reactive protein.

1. Introduction

As a common metabolic disease, type 2 diabetes mellitus (T2DM) is complicated by many cardiovascular diseases and cerebrovascular diseases, such as peripheral vascular diseases, heart failure, and intracerebral hemorrhage [1,2]. Compared with non-diabetic patients, diabetic patients are at a higher risk of hemorrhagic stroke [2]. Intracerebral hemorrhage (ICH) can cause considerable damage to the central nervous system with a high rate of disability and mortality [3]. Thus, the risk factors and pathogenesis of ICH in T2DM patients attract increasing attention.

The mechanism of ICH in T2DM remains unclear. However, studies have demonstrated that the risk of ICH is increased with chronic inflammation [4] and that diabetic patients exhibit long-term chronic inflammation [5,6]. It is easy and effective to obtain the neutrophil to lymphocyte ratio (NLR), an index of systemic inflammation [7]. Its prognostic value in tumors [8] or cardiovascular diseases [9] has been suggested by recent studies. Its association with diabetic complications has gradually received attention [10-13]. Elevated NLR is associated with risk factors of ICH, including atherosclerosis [14] and hypertension [15]. Therefore, elevated NLR itself may be used as an alternative marker to predict the pathogenesis of ICH.

The present study was designed to explore the correlation between NLR and ICH in T2DM patients and the independent risk factors of ICH in T2DM patients. This information will assist patients in taking early precautions to reduce the impact of ICH on both the health and wealth of T2DM patients.

2. Methods

2.1. Subjects

All procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975 as revised in 2008. The local ethics committees approved the study protocol. In this study, 1259 diabetes patients admitted to Zhujiang Hospital and Chinese PLA General Hospital between January 2008 and December 2014 for their primary diseases were retrospectively evaluated using the electronic medical record system. Of these patients, 848 were newly diagnosed with Type 2 Diabetes Mellitus and were included for further exclusion. Type 2 Diabetes Mellitus was diagnosed based on the American Diabetes Association consulting criteria (i.e.,

fasting plasma glucose [FPG] of ≥7.0 mmol/L [126 mg/dL] and/or a 2-h post-glucose value of ≥11.1 mmol/L [200 mg/dL]).

Of these 848 patients, patients who matched the following exclusion criteria were excluded: cardiovascular diseases, myocardial infarction, heart failure, active infection, active massive hemorrhage, acute poisoning, cancer or blood diseases that affect neutrophils or lymphocytes (e.g., myeloproliferative diseases and leukemia), ICH before admission or caused by other confirmed reasons after discharge (trauma, drugs, congenital abnormalities, coagulation disorders, vasculitis, brain tumor, vascular amyloidosis, or hemorrhage secondary to ischemic stroke), or taking medication that affects neutrophils and lymphocytes (chemotherapy or radiotherapy to malignancy, granulocyte colony stimulating factor therapy or corticosteroid therapy).

After the second exclusion, 429 patients were included. Phone follow-up was implemented by using the patient database of Zhujiang Hospital and Chinese PLA General Hospital. Regarding the 75 patients who were diagnosed with ICH at Zhujiang Hospital and Chinese PLA General Hospital and 12 patients who were diagnosed with ICH at other hospitals, the patients or their family members were required to return to the hospital for a follow-up visit and information supplement. All 87 ICH patients were categorized into the ICH group. In total, 342 patients whose age and gender matched the criteria were categorized into the control group.

We followed the ADA guidelines [16] to treat T2DM and AHA/ASA guidelines [17] to treat intracerebral hemorrhage. For other complications, patients were provided standard symptomatic treatment.

2.2. Data collection

After a minimum 8-hour fast, the systolic and diastolic pressure of all patients were measured by standardized mercury sphygmomanometer (XJ11D, Shanghai Medical Instruments Co., China). Height and weight were also measured to calculate the body mass index (BMI) as weight divided by height squared (kg/m^2). A venous blood sample was obtained from the ulnar vein of each patient after clinical measurements of blood pressure, height and weight. Laboratory tests, including HbA1c, fasting glucose, fasting insulin, creatinine, uric acid, triglyceride, total cholesterol, HDL and LDL, were conducted.

The daily urine of each patient was collected to measure urinary micro albumin using a turbidimetric immunoassay (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Fasting glucose was measured using the glucose oxidase method. Fasting insulin was measured by the chemiluminescence method. An automated biochemical analyzer Synchron CX5 (*Beckman* Instruments Inc., Brea, USA) was used to measure triglyceride, total cholesterol, HDL and

LDL. HbA1c was measured using an automated high performance lipid chromatography Tosoh G7 (Tosoh Europe N.V, Tessenderlo, Belgium). Insulin resistance (IR) was assessed with a homeostasis model. HOMA-IR is FPG (mmol/L) ×FIN (mU/L)/22.5 [18].

2.3. Definitions

Intracerebral hemorrhage (ICH) was defined as a stroke for which CT scanning can identify an area of high density within the brain parenchyma with or without extension into the ventricles or subarachnoid space or an area of attenuation with ring enhancement after injection of contrast for scans performed after 1 week. MRI typically reveals an area of hypointensity or isointensity on T1-weighted images or an area of marked hypointensity on T2-weighted images. Alternatively, the origin of the hemorrhage can be demonstrated by investigation at autopsy of the cerebral parenchyma [19]. Type 2 diabetes mellitus was defined as fasting serum glucose of ≥ 7.0 mmol/L and/or non-fasting serum glucose of ≥ 11.1 mmol/L [20]. The neutrophil-to-lymphocyte ratio (NLR) was defined as a ratio of the neutrophil and lymphocyte counts. Current smoking was defined as a patient who had smoked 100 cigarettes before and smoked every day or every few days prior to admission and diagnosis of diabetes [21]. Hypertension was defined as an average SBP ≥ 140 mmHg or DBP ≥ 90 mmHg and/or current use of antihypertensive medication prescribed by a physician [22].

2.4. Statistical analysis

Statistical analysis was performed by using SPSS 16.0 (SPSS, Chicago, Illinois, USA). Continuous variables were represented as the means ± SD or medians and interquartile range. Categorical variables were expressed as percentages. Means for continuous variables between groups were compared using student's t test, the Mann-Whitney U or Bonferroni-corrected Mann-Whitney U test, ANOVA, or the Kruskal-Wallis test, when necessary. Categorical variables were compared using the χ^2 test. Pearson's correlation analysis was conducted to determine the correlation between NLR and ICH. Logistic regression analysis was used to identify the effect of each factor on ICH. Receiver operating characteristic analysis was applied to obtain the cut-off level of elevated NLR to predict ICH. A P-value of < 0.05 was considered statistically significant, and the confidence interval was defined as 95%.

2.5. Results

A population of 429 patients was included in this retrospective study. The mean age of these patients was 63.67 ± 9.74 years, and males constituted 43% of the patients. All patients were divided into two groups depending on the presence of cerebral hemorrhage: the cerebral hemorrhage group (n=87) and the control group (n=342). All patients were also categorized into quartiles by NLR. The clinical characteristics and laboratory parameters of the study population are listed in Tables 1 and 2. Patients in both groups were matched in terms of age

(P=0.416) and gender (P=0.057). In addition, no significant differences in insulin resistance, BMI, HbA1c, FPG, FINS, micro albumin, uric acid, total cholesterol, triglyceride, HDL, LDL and current smoking were noted. Significant differences were identified in systolic blood pressure. The laboratory characteristics are also presented in **Table 1**. White blood cell count, neutrophil count, lymphocyte count, neutrophil-to-lymphocyte ratio and creatinine were significantly different between the two groups.

In the Pearson's correlation analysis, elevated NLR was significantly correlated with ICH (r=0.296, P<0.001). A multivariate logistic regression model was constructed to identify the independent predictor of ICH in diabetic patients. Elevated NLR acquired when diabetes was newly diagnosed was independently associated with ICH after discharge from the hospital (OR=3.893, 95% CI 2.324-6.521, P<0.001). The adjusted OR of NLR was more obvious than current smoking (OR=2.729, 95% CI 1.453-5.127, P=0.002) and systolic blood pressure (OR=1.018, 95% CI 1.003-1.034, P=0.022) (**Table 3**).

ROC analysis was performed to determine the cut-off of NLR, WBC, neutrophil count and lymphocyte count to predict ICH in diabetic patients, as shown in Figure 1. An NLR of >2.58 may be used to predict the ICH with a sensitivity of 0.69 and specificity of 0.66 (area under the curve is 0.72, 95% CI 0.659-0.780, P<0.001). The cut-off of the remaining parameters is presented in **Table 4**.

2.6. Discussion

Diabetic patients are at high risk of atherosclerosis and corresponding complications, including coronary artery diseases, acute coronary syndrome and stroke (both ischemic and hemorrhagic) [23,24]. The diagnosis of ICH depends on tomographic instruments, including CT or MRI, to determine whether and where hemorrhage has occurred. Alternately, a diagnosis might be made according to symptoms and physical signs [25]. Remedial work could only be conducted after the occurrence of ICH, indicating that such patients must suffer from the consequences of the ICH. Therefore, research on a new marker capable of predicting ICH and preventing adverse outcome is important.

Abnormal blood sugar in diabetic patients can lead to the change of NLR which represent the long-term low-grade inflammation. This phenomenon illustrate how diabetes contributes to atherosclerosis and increase the risk of ICH.

Neutrophils represent the active nonspecific inflammatory mediator initiating the first line of defense, whereas lymphocytes represent the regulatory or protective component of inflammation [26]. Chronic hyperglycemia can promote the rise of granulocyte count by secretion of a large number of inflammatory factors and inflammatory cytokines and decrease the lymphocyte count. Meiqin Lou et al [27]'s study had shown that the NLR values of the

diabetic patients were significantly higher than those of the healsthy control. Khodabandehlou T et al [28] found that lymphocyte levels were reduced as a result of hyperglycemia in patients with diabetes and healthy subjects. And patients with diabetes mellitus have been suggested to have insufficient proliferation of lymphocytes [29] in one study by Chang FY and Shaio MF. The reduce the number of CD8+ T cells, inhibiting the anti-inflammatory environment [30], makes the persistent low-grade inflammation and a vicious cycle.

Inflammation plays an important role in the pathogenesis of atherosclerosis [31,32]. Numerous types of inflammatory factors, such as interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1), and intercellular adhesion molecule-1 (ICAM-1), exist in the development of atherosclerotic plaques in the endothelium of vessels [33,34]. Previous studies have noted that during the development of atherosclerosis, blood neutrophil count increases, and neutrophils are attracted into the plaque to excrete pro-inflammatory products, such as elastase, myeloperoxidase, reactive oxygen species, leading to continual damage to the vessel walls [35] and impairment of endothelial function. In addition, the advanced glycation end products (AGEs) are characteristic of pro-inflammatory and potentially atherogenic functions [36,37]. Thus, the predictive value of NLR in atherosclerosis has been suggested by many studies [14, 38,39].

Through atherosclerosis and damaging endothelium of vessels, the NLR was directly and significantly associated with the risk of ICH in T2DM patients and the index also represents a reliable and dynamically stable marker of systemic inflammation that reflects the immune response and combines information of innate and adaptive pathways in a long duration.

Romero JR. et al [40] find out that carotid atherosclerosis which happened at the internal carotid artery was associated with increased cerebral microbleed (CMB)-hemorrhage-prone small vessel disease, mainly in deep regions, and the association was stronger if atherosclerosis is more severe. Common carotid artery (CCA) atherosclerotic changes may result in lower risk of hemorrhages represented by CMB due to release of prothrombotic inflammatory/endothelial cytokines.

On the other hand, recent research has proved that the NLR can independently contribute to hypertension [41], which is the most significant risk factor of hemorrhagic stroke [42]. Hypertension causes damage to the endothelium of small arteries, leading to atherosclerosis [43] and remodeling of the cerebral vasculature. Thus, hypertension causes microaneurysms at the bifurcation of arterioles. Chronic elevation of intraluminal arterial pressure can damage small vessel wall, thereby leading to eventual disruption [44]. Recent research has demonstrated that chronic low-grade inflammation plays an important role in the pathogenesis of hypertension. For example, inflammation causes endothelial dysfunction through producing NO,

which leads to a disorder between vasodilation and vasoconstriction. Thus, oxidative stress and inflammation are increased. NO production may subsequently contribute to the development of hypertension [45]. Several studies have shown that the adaptive immune response in particular contributes significantly to the pathophysiology of hypertension [46].

As a novel inflammatory marker, NLR can be acquired from routine blood counts after admission. Given its predictive value and convenience, NLR can be envisaged to be employed in screening those T2DM patients at high risk of primary ICH in the future and more potential inflammatory marker should be discussed, such as IL-6, IL-8 et al.

3. Conclusion

We conclude that as a systematic inflammation marker, NLR may be an independent risk factor of ICH. An elevated NLR can be used to predict the risk of ICH, which is helpful for the prevention of ICH. Further large-scale and long-term clinical trials are necessary to support our findings.

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5. Authors' Contributions

All authors with the same contribution to the study were responsible for the study design, data collection, and manuscript writing. Li Rui and Yue Shufan helped with the acquisition and interpretation of data and with manuscript revisions. Luo Peng analyzed the data, guaranteed this work, provided academic guidance, and took responsibility for the accuracy of the data analysis

6. Statement of Human and Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

7. Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study.

8. Tables

Table 1: Comparison of baseline characteristics of the study population

	Control group(n=342)	ICH group(n=87)	Overall p value
Age (year)	63.86±10.31	62.92±7.06	0.320
Gender (male,%)	43%	46%	0.582
Insulin resistance	4.03±4.56	4.39±4.10	0.499
White blood cell count (10^9/L)	6.08±1.59	6.60±1.42	0.006
Neutrophil-to-lymphocyte ratio	2.30±0.58	2.74±0.55	<0.001
Neutrophil count (10^9/L)	3.77±0.99	4.11±0.93	0.004
Lymphocyte count (10^9/L)	1.71±0.51	1.53±0.35	<0.001
Platelet count (109/L)	220.9058.55	224.3556.06	0.621
Body mass index (kg/m^2)	24.34±3.65	24.66±3.57	0.462
Systolic blood pressure (mmHg)	132.35±18.54	143.20±20.84	<0.001
Diastolic blood pressure (mmHg)	78.01±9.96	82.25±11.18	0.001
Hypertension (%)	106(31%)	56(64%)	<0.001
HbA1c (%)	7.36±1.74	7.56±1.74	0.336
Fasting glucose (mmol/L)	8.52±3.95	7.97±3.82	0.247
Fasting insulin (mU/L)	11.01±9.86	12.76±11.24	0.152
Micro albumin in uria (mg/24h)	23.39±33.21	23.33±36.65	0.989
Creatinine (μmol/L)	81.13±18.81	80.90±15.41	0.916
Uric acid (μmol/L)	299.28±110.74	330.01±114.50	0.022
Total cholesterol (mmol/L)	5.24±1.24	5.48±1.54	0.127
Triglyceride (mmol/L)	2.00±1.04	2.72±1.38	<0.001
HDL (mmol/L)	1.34±0.23	1.38±0.40	0.194
LDL (mmol/L)	2.82±0.91	2.89±0.66	0.549
Current smoker (%)	48(14%)	23(26%)	0.017
Hypersensitive C-reactive protein (mg/L)	20.5±15.3	21.3±17.5	0.074
Comorbidities (%)			
Pulmonary infection	54(16%)	42(48%)	<0.001
Upper gastrointestinal hemorrhage	43(13%)	29(33%)	<0.001
Urinary tract infection	27(8%)	8(9%)	0.692
Diabetic retinopathy	10(3%)	3(3%)	0.987
Diabetic nephropathy	21(6%)	6(7%)	0.795
Drug treatment (%)			
Oral hypoglycemic agents	283(83%)	65(75%)	0.087
Antihypertensive	179(52%)	48(55%)	0.618
Antithrombotic	106(31%)	32(37%)	0.302
Stain	89(26%)	26(30%)	0.467
Duration of T2DM (Years)	9.6±4.4	8.7±6.2	0.120

Table 2: Participant characteristics by quartiles of neutrophil/lymphocyte ratio

Quartiles of neutrophil/lymphocyte ratio(range)					
	Level1	Level2	Level3	Level4	P value
	(0.21-1.94)	(1.95-2.42)	(2.44-2.88)	(2.90-8.72)	
	n=107	n=107	n=113	n=102	
Age(year)	62.76±10.65	63.52±9.93	63.48±9.31	64.98±8.98	0.416
Gender(male,%)	71(66.4%)	54(50.5%)	64(56.6%)	53(51.9%)	0.057
Prevalence of ICH	15(14%)	19(18%)	19(18%)	34%(31%)	0.034
Insulin resistance	3.73±3.93	3.79±3.02	4.61±6.54	4.27±3.29	0.418
Whitebloodcellcount(109/L)	5.56±1.46	6.09±1.40	6.46±1.75	6.65±1.42	<0.001
Neutrophilcount(109/L)	1.58±0.28	2.19±0.14	2.65±0.14	3.16±0.18	<0.001
Lymphocytecount(109/L)	3.14±0.84	3.80±0.83	4.028±0.96	4.41±0.86	<0.001
Platelet count(109/L)	223.66±58.56	222.94±56.79	221.73±57.09	217.88±60.35	0.894
Bodymassindex(kg/m2)	2.03±0.61	1.74±0.38	1.5193±0.36	1.39±0.27	0.958
Systolicbloodpressure(mmHg)	24.45±3.79	24.37±3.80	24.28±3.37	24.54±3.60	<0.001
Diastolicbloodpressure(mmHg)	128.56±15.62	134.34±18.67	135.19±19.41	140.33±22.33	0.001
HbA1c(%)	7.55±0.79	8.06±1.15	7.90±0.97	8.05±1.12	0.911
Fastingglucose(mmol/L)	7.36±1.68	7.33±2.00	7.49±1.60	7.43±1.66	0.462
Fastinginsulin(mU/L)	8.06±3.54	8.85±4.16	8.50±4.18	8.19±3.75	0.47
Microalbumininuria(mg/24h)	10.77±10.61	10.48±8.87	11.78±12.11	12.46±8.51	0.102
Creatinine(μmol/L)	76.25±17.50	81.14±18.45	81.91±16.69	85.15±19.15	0.005
Uricacid(μmol/L)	291.25±105.06	304.16±115.89	309.46±105.80	317.51±121.43	0.384
Totalcholesterol(mmol/L)	5.29±1.43	5.19±1.00	5.23±1.012	5.44±1.70	0.53
Triglyceride(mmol/L)	2.12±1.16	2.04±1.04	2.05±1.14	2.41±1.24	0.068
HDL(mmol/L)	1.34±0.22	1.34±0.24	1.33±0.23	1.36±0.38	0.83
LDL(mmol/L)	2.718±0.88	2.90±0.72	2.81±0.91	2.92±0.94	0.299
Current smoker(%)	19(17.7%)	18(16.8%)	20(17.7%)	14(13.7%)	0.847

Table 3: Independent predictor of ICH in multivariate logistic regression analysis

	Unadjusted OR(95%CI)	**P value**	**Adjusted OR(95%CI)**	**P value**
White blood cell count (109/L)	1.224(1.059-1.416)	0.006	1.059(0.891-1.259)	0.516
Neutrophil-to-lymphocyte ratio	4.135(2.567-6.662)	<0.001	3.717(2.216-6.233)	<0.001
Current smoker (%)	2.201(1.250-3.876)	<0.001	2.850(1.502-5.407)	0.001
Systolic blood pressure (mmHg)	1.027(1.015-1.039)	0.001	0.995(0.973-1.017)	0.632
Diastolic blood pressure (mmHg)	1.040(1.016-1.064)	0.006	1.011(0.981-1.041)	0.472
Hypertension (%)	3.967(2.419-6.507)	<0.001	3.618(1.565-8.362)	0.003

Table 4: Receiver operating characteristic (ROC) analysis for neutrophil-to-lymphocyte ratio to predict ICH (area under curve is 0.720)

Parameters	AUC	Cut-off value	Sensitivity	Specificity	Youden index
WBC	0.619	6.350	0.586	0.655	1.241
NLR	0.720	2.579	0.690	0.664	1.354
Neutrophil	0.608	3.750	0.667	0.515	1.182
Lymphocyte	0.400	1.797	0.202	0.620	0.827

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