ORIGINAL ARTICLE

Complete Blood Count Parameters for Prediction of non-ST Segment Elevation Myocardial Infarction

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Abstract

Objective: To evaluate whether the complete blood count parameters can be used to predict patients who will have positive troponin levels during emergency department observation, and to establish whether any single parameter or combination of parameters has sufficiently good diagnostic test criteria results to be recommended for use in daily clinical practice.

Study Design: An observational study.

Place and Duration of Study: Department of Emergency Medicine, Duzce University School of Medicine, Duzce, Turkey, from October 2015 to October 2016.

Methodology: Study group had patients with positive troponin levels during observation in the emergency department. The control group had normal troponin levels. Their complete blood count parameters were compared individually and in combination.

Results: Total white blood cell count, neutrophil count, red cell distribution width, neutrophil-to-lymphocyte ratio, and some combinations of these parameters were found to be predictive of troponin elevation. The best one was combination of white blood cell count, red cell distribution width and neutrophil-to-lymphocyte ratio.

Conclusion: Some of the complete blood count parameters may provide some clues when predicting troponin elevation in patients with chest pain. However, none of these parameters or no combination of them have sufficiently good diagnostic test criteria results to safely predict non-ST segment elevation myocardial infarction.

Key Words: *Complete blood count, Myocardial infarction, Neutrophil-to-lymphocyte ratio, Red cell distribution width, Troponin, White blood cell count.*

INTRODUCTION

Rapid diagnosis or exclusion of acute coronary syndromes (ACS) in patients reporting chest pain is important while determining the appropriate approach. Elevation of serum levels of myocardial necrosis markers (creatine kinase, creatine kinase muscle-brain isoband and troponins including high-sensitivity troponin) currently used for diagnosis of non-ST segment elevation myocardial infarction (NSTEMI) takes several hours.[1] Finding new markers which can help physicians predict cardiac marker elevation earlier would be valuable. At this point, complete blood count (CBC) appears as a simple, economical, and widely available test which may have some potential to be used for this purpose. Though, the CBC has such advantageous features, parameters included in this test should have rational relations with pathophysiology of ACSs so that physicians can use them to predict NSTEMI. Neutrophils increase in number and become activated in the case of an ACS.[2,3] Neutrophils and platelets have mutual interaction, and neutrophils contribute to the damage in the lesion site by both classic recruitment cascade and adhering to platelets attached to endothelial cells.[4] Briefly, systemic inflammation modulates the coagulation process,[5] and various CBC parameters were defined as useful predictive biomarkers in cardiovascular events.[6-9] Single use of either is limited by their low sensitivity and specificity, and we do not have definitive information whether CBC parameters may help distinguish patients having positive troponin levels during observation in the emergency department (ED) from those with normal levels.

Hence, the present study had objectives of evaluation whether CBC parameters can be used to predict patients who will have positive troponin levels during observation in ED; to establish whether combinations of these markers are more effective predictors of troponin elevation compared with a single marker, and if any single parameter or combination of more than one parameters has sufficiently good diagnostic test criteria (DTC) results to be recommended for use in clinical practice.

METHODOLOGY

This study was conducted as a prospective, single-centre, observational study from October 15th, 2015 to October 14th, 2016 at Department of Emergency Medicine,

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Duzce University School of Medicine, Duzce, Turkey. Of the 1,241 consecutive patients presenting to the ED due to chest pain within the first 12 hours of symptom onset, 711 were excluded according to exclusion criteria; remaining 530 patients meeting the inclusion criteria and not having any exclusion criterion were recruited into the study. Of these 530 patients, 120 were included in the study group (patients having positive troponin levels during observation) and remaining 410 patients in the control group (those with normal troponin levels). Inclusion criteria were chest pain which does not fit the criteria for unstable angina (UA) pectoris and absence of ST elevation or new left bundle branch block on the electrocardiogram (ECG). A resting ECG was taken within the first 10 minutes of admission to the ED. A second ECG was taken within 30 minutes, if the first ECG was normal or inconclusive. Later, it was repeated every three hours, and immediately in the case of recurrence or aggravation of the symptoms. Exclusion criteria were clinically significant valvular heart disease, supraventricular/ ventricular tachycardia, implantable cardioverter defibrillator shock, cardiomyopathy, myocarditis, pulmonary embolism, cerebrovascular incident, significant congestive heart failure, hematologic disease, cancer, significant kidney or liver disease, suspicion/ presence of continuing infection or systemic inflammatory condition, autoimmune disease, thrombocytopenia and anticoagulant or steroid use. All participants gave written informed consent. The study was approved by local Research Ethics Committee.

Blood samples for troponin were taken at the time of presentation and then every 4 to 6 hours for 12 to 24 hours observation. Measurement of CBC parameters including total white blood cell count (WBC) and differential leukocyte counts, hemoglobin (Hb), mean platelet volume (MPV), red cell distribution width (RDW) and platelet distribution width (PDW) were conducted in peripheral blood samples collected on admission. CBC parameters were measured by an automated hematology analyser (Beckman Coulter LH 780; Beckman Coulter, Fullerton, California). Absolute cell counts were used in the analyses, and the neutrophil-to-lymphocyte ratio (NLR) was calculated as the ratio of absolute number of neutrophils to that of lymphocytes. At least two troponin tests were performed using the Advia Centaur TnI-Ultra assay (Siemens Healthineers, Tarrytown, NY, USA).

In this study, categorical variables were expressed as frequencies along with percentages and continuous variables were presented as median (Interquartile Range-IQR) values. The Kolmogorov-Smirnov test was used to evaluate whether the distribution of continuous variables was normal. Groups were compared with Mann-Whitney U-test. The Receiver Operating Characteristics (ROC) curve analysis was used to determine the best cut-off value of CBC parameters and sensitivity analysis. DTC results (sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio) for combined use of more than one CBC parameters were calculated using Boolean Algebra and Logistic Regression model. Categorical variables were compared with Pearson Chi-square test. The Statistical Package for the Social Science for Windows version 22 (SPSS Inc., Chicago, Illinois) was used for most calculations, and Boolean Algebra calculations were performed manually. A p-value below 0.05 was considered to be significant.

RESULTS

Mean age of the patients was 60.1 ±13.1 years, and 37% (n=196) of the participants were females; 63% (n=334) were males. The study and control groups were homogenous with respect to age and gender (p=0.052 and p=0.727, respectively).

WBC, RDW, neutrophil count and the NLR values were significantly higher in the study group compared to the control group (p<0.001, p=0.020, p<0.001, and p<0.001, respectively). However, there were no significant differences between the groups regarding MPV, PDW, lymphocyte count and Hb levels (p=0.179, p=0.519, p=0.326, p=0.695, respectively). Descriptive statistics of the CBC parameters are given in Table I.

ROC analysis was performed to determine the best cut-off values for WBC, RDW, neutrophil count and NLR, which were found to differ significantly between the groups. DTC results of those parameters are given in Table II. The NLR only had both sensitivity and specificity above 60% (61% and 62%, respectively). Although, statistically significant cut-off values could be found for each of these tests, combined use of them has been evaluated because diagnostic test criteria (DTC) results of none of these parameters were satisfactory.

CBC parameters with statistically significant cut-off values were transformed into binary form (study *vs*. control). Combinations of those transformed variables were evaluated. Table III includes DTC results for combined use of these transformed variables. Combination of WBC, RDW and NLR had the highest sensitivity and the

Table I: Comparison of the groups regarding descriptive features of complete blood count parameters.

	Control group (n=410) Median (IQR)	Study group (n=120) Median (IQR)	p
WBC	8.2 (2.7)	8.9 (3.3)	<0.001
RDW	13.7 (1.5)	14.1 (1.7)	0.020
MPV	8.45 (1.4)	8.4 (1.3)	0.179
PDW	16.8 (0.9)	16.7 (0.8)	0.519
Lymph	2.3 (1.3)	2.2 (1.1)	0.326
Neutr	4.6 (2.2)	5.7 (2.9)	<0.001
NLR	1.9 (1.3)	2.6 (1.9)	<0.001
Hb	13.6 (2.1)	13.6 (2.3)	0.695

IQR: Interquartile Range, WBC: White blood cell count, RDW: Red cell distribution width, MPV: Mean platelet volume, PDW: Platelet distribution width, Lymph: Lymphocyte count, Neutr: Neutrophil, IQR count, NLR: Neutrophil-to-lymphocyte ratio, Hb: Hemoglobin.

Table II: Diagnostic test criteria results of complete blood count parameters with statistically significant cut-off values.

	Cut-off value	Sensitivity	Specificity	LR(+)	LR(-)	PPV	NPV	AUC- ROC	SEM	p
WBC	10.4	0.32	0.88	2.71	0.78	0.41	0.83	0.61	0.03	<0.001
RDW	13.7	0.66	0.51	1.33	0.68	0.25	0.85	0.57	0.03	0.020
Neutr	5.7	0.52	0.74	2.03	0.65	0.34	0.86	0.65	0.03	<0.001
NLR	2.34	0.61	0.62	1.59	0.63	0.28	0.85	0.61	0.03	<0.001

WBC: White blood cell count, RDW: Red cell distribution width, Neutr: Neutrophil count, NLR: Neutrophil-to-lymphocyte ratio LR(+): Positive likelihood ratio, LR(-): Negative likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, AUC-ROC: Area under the ROC curve, SEM: Standard error of mean.

Table III: Diagnostic test criteria results for combined use of complete blood count parameters with statistically significant cut-off values.

TESTS	Sensitivity	Specificity	LR(+)	LR(-)	PPV	NPV	AUC- ROC	SEM	p
WBC-RDW	0.27	0.94	4.50	0.78	0.82	0.56	0.62	0.03	<0.001
Neutr-RDW	0.38	0.85	2.53	0.73	0.72	0.58	0.66	0.03	<0.001
NLR-RDW	0.43	0.76	1.79	0.75	0.64	0.57	0.65	0.03	<0.001
Neutr-WBC	0.31	0.89	2.82	0.78	0.74	0.56	0.64	0.03	<0.001
NLR-WBC	0.24	0.93	3.43	0.82	0.77	0.55	0.65	0.03	<0.001
NLR-Neutr	0.43	0.80	2.15	0.71	0.68	0.58	0.66	0.03	<0.001
WBC-RDW-NLR	0.57	0.71	1.97	0.61	0.66	0.62	0.67	0.03	<0.001

WBC: White blood cell count, RDW: Red cell distribution width, Neutr: Neutrophil count, NLR: Neutrophil-to-lymphocyte ratio LR(+): Positive likelihood ratio, LR(-): Negative likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, AUC-ROC: Area under the ROC curve, SEM: Standard error of mean.

area under the ROC curve (AUC-ROC) value among all combinations (0.57 and 0.67, respectively). Besides, logistic regression model showed that WBC above 10.4 increases the risk of the patient to have troponin elevation 2.64 times (95% CI: 1.58%-4.40%, p<0.001for Odds Ratio [OR]); RDW above 13.7 has an OR of 1.73 (95% CI: 1.11%-2.69%, p=0.016 for OR), and NLR above 2.34 means 2.11 times increased risk (95% CI: 1.37%-3.25%, p=0.001 for OR).

DISCUSSION

Identification of a new biomarker indicating myocardial ischemia before the elevation of troponins would be of significant clinical value because of the limited sensitivity of troponins in the early phase of acute myocardial infarction.[1] Several markers of vascular inflammatory processes or oxidative stress, such as myeloperoxidase, elastase, CD40/CD40 ligand, ischemia-modified albumin and lipoprotein-associated phospholipase A2 have been evaluated in patients with acute chest pain.[1,9] However, those tests are not widely available and are expensive so it was aimed, in the present study, to find out cheaper and widely available alternatives. CBC parameters, which are available world-wide and ordered routinely even in developing and non-developed countries, where most of the people in the world live, were evaluated for predictive value in the estimation of troponin elevation, and some clues have been found indicating that WBC, neutrophil count, RDW and NLR may provide some guidance.

The NLR was the only parameter with both sensitivity and specificity above 60%. It was previously defined as the best predictive marker of cardiovascular events among all CBC parameters.[10] This simple index represents the balance between neutrophils, the active inflammatory component, and lymphocytes, the regulatory and protective component.[11] Therefore, a higher NLR indicates a greater level of inflammation.[10] Increased neutrophils are associated with the formation of platelet-leukocyte aggregates in the vascular lumen, which leads to the extension of infarct area in patients with an ACS.[12] The NLR values were found to be significantly higher in the study group compared to the control group in the present study but physicians cannot decide whether troponin elevation will ensue by solely relying on this parameter. The literature includes a few studies evaluating the use of the NLR in prediction of ACSs.[6,9,13,14]

Yilmaz *et al*. reported that the NLR is an independent predictor of coronary thrombus formation in patients with non-ST segment elevation ACSs (NST-ACS) (sensitivity=93%; specificity=62%, AUC-ROC=0.86).[6] The AUC-ROC for the NLR was 0.61 in the current study. The blood sample for CBC was collected one hour after admission in the study by Yilmaz *et al*.[6] but they were taken on admission in the present study. The NLR may change significantly in an hour due to ongoing inflammatory process, and an apparent difference may develop between the groups because inflammatory response in the group with thrombus formation is more prominent. Though UA patients were compared with NSTEMI patients in the study by Yilmaz *et al*.,[6] better sensitivity of, and higher AUC-ROC for the NLR might be caused by this difference in the timing of blood sampling.

Korkmaz *et al*. reported that higher admission NLR values independently predict the patients in whom troponin became positive during observation (sensitivity=79%; specificity=73%; AUC-ROC=0.83).[9] Methodologic variations, such as differences in the exclusion criteria, may explain the differences in DTC results of the NLR between the study by Korkmaz *et al*.[9] and the current study. Steroid use, which was not an exclusion criterion in the study by Korkmaz *et al*.,[9] may be an example of differences in the exclusion criteria. Steroid use significantly affects inflammatory response and may lead to apparently different NLR values.[10]

Ozturk *et al*. found that NLR levels were higher in NST-ACS than in the control group.[13] That study differs from the present study in some respects: It was a retrospective study; patients under the age of 45 only were recruited; the sample size was quite low (44 patients with UA, 40 with NSTEMI and 40 controls, 124 participants totally), and the NLR was not evaluated for DTC. Zazula *et al*. found that patients diagnosed with non-cardiac pain had the lowest NLR, followed by the UA group and the NSTEMI group, and the highest value was observed in the STEMI group.[14] The NLR was reported to have 31% sensitivity; AUC-ROC was 63%. They have found that sensitivity of the NLR for detection of NST-ACS (UA and NSTEMI) was still low (23%) after 35 patients who had been diagnosed with STEMI were excluded, and they have concluded that its low sensitivity could not be diagnostically reliable if used in isolation. That study also had some methodologic differences compared to the current study. Troponin levels were not calculated, and UA and NSTEMI patients were included in the same group (NST-ACS group). The latter may explain why the sensitivity of NLR in predicting NST-ACSs was found to be significantly lower in this study compared to the current one (23% *vs*. 61%) because inflammatory response is expected to be less prominent in patients with UA compared to those with NSTEMI.

In the current study, WBC, neutrophil count and RDW values, in addition to the NLR, were found to be significantly higher in the study group compared to the control group. There are several studies in the literature reporting a relation between ACSs and CBC parameters together with or other than the NLR.[6,9,13,14]

Yilmaz *et al*. reported that the patients with higher neutrophil counts and lower lymphocyte counts showed increased thrombus incidence (NSTEMI) among the patients with NST-ACS.[6] Korkmaz *et al*. found that the patients in the troponin-positive group had higher WBC, and neutrophil counts; but lower lymphocyte counts than the troponin-negative group did.[9] Ozturk *et al*. reported that percent of neutrophils was higher in the NST-ACS group than in the control group.[13] Zazula *et al*. found that WBC and neutrophil count were lower in the patients with non-cardiac pain, intermediate in those with UA and NSTEMI, and the highest in the patients with STEMI.[14] However, lower numbers of lymphocytes were observed in the STEMI group, followed by NSTEMI group and the highest levels were seen in the non-cardiac pain group. Although these studies have some methodologic differences compared to the present study, the results support those of the current study because WBC and neutrophil counts were found to be higher in the study group in the current study, too. However, lymphocyte counts did not significantly differ between the groups in contrast to the results of the studies by Yilmaz *et al*., Korkmaz *et al*. and Zazula *et al*.[6,9,14]

Some platelet parameters, also, are included in CBC, and it is known that platelet activation is a link to thrombosis and inflammation.[15] Numerous platelet markers, including MPV and PDW have been investigated in connection with both thrombosis and inflammation.[12] The MPV is a marker of platelet reactivity, and was found to be independently associated with carotid atherosclerosis and increased acute coronary event risk.[7,16,17] The PDW was reported to be able to predict coronary total occlusion.[18] Whereas, no significant difference was found between the groups in MPV or PDW values in the present study. Korkmaz *et al*., too, reported that MPV did not differ significantly between the groups with and without troponin elevation.[9]

RDW, another CBC parameter, has been found to be associated with adverse outcomes in CAD.[19] The RDW has been described to be a stronger biomarker for coronary heart disease death than widely used inflammatory markers like hsCRP.[20] Korkmaz *et al*. found that patients having troponin elevation had higher RDW values compared to those with normal levels.[9] The results of the present study are similar; however, it is suggested that solitary use of this parameter in prediction of NSTEMI may be misleading because its DTC results were quite poor.

Because the DTC results of none of the CBC parameters discussed untill now were good enough to be recommended for solitary use in prediction of NSTEMI, combined use of these parameters have been evaluated. Combination of WBC, RDW and NLR was seen to have the highest sensitivity and specificity. However, both sensitivity and specificity of this combination are still low for routine use in clinical practice. But, it was also seen that RDW or NLR above the cut-off value increases the risk of the patient having troponin elevation nearly twice, and a WBC above the cut-off value increases the risk more than two-and-half times. RDW and NLR above the cut-off values were, also, found to show increased risk of troponin elevation in the study by Korkmaz *et al*.[9]

This study has some limitations: The cross-sectional design and the lack of in-hospital and long-term follow-up are the major ones; and the lack of simultaneous measurement of other inflammatory markers and comparison with CBC parameters are other limitations. Finally, having no information about the basal levels of CBC parameters before the index event, is also a limitation.

CONCLUSION

None of the CBC parameters or no combination of them have sufficiently good DTC results to safely predict NSTEMI. Besides, there might be some sorts of selection bias in some of the previous studies reporting shinier results about the use of CBC parameters for

prediction of ACSs. Finally, there is still a need to find out new tests which can predict NSTEMI earlier than the troponins can do.

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