

DIAGNOSTIC UTILITY OF LEUKOCYTE PARAMETERS IN THE PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background. Inflammation is one of the key players in acute myocardial infarction (AMI). One of the ways to evaluate it indirectly is to analyze leukocyte parameters of complete blood count (CBC), which is a routine and affordable method of diagnosis. Leukocyte counts can provide additional information about the course, as well as a potential prognosis for complications of AMI. We suggest that the dynamic changes of CBC during the treatment of the patients with AMI may be of value to assess the prognosis of the course of the disease, and therefore require further study.

Objective. The aim of the study to evaluate the diagnostic and prognostic potential of leukocyte indexes of CBC, in particular the levels of leukocytes, lymphocytes, neutrophils and N/L, WBC/MPV, PLT/L ratios in the patients with AMI at the time of hospitalization and on the 7th day of hospital stay.

Methods. The study involved 204 individuals: 152 patients with AMI (Group 1), 30 patients with stable coronary heart disease (Group 2) and 24 healthy volunteers (Group 3). Hemogram parameters and their ratios, in particular the levels of leukocytes, lymphocytes, neutrophils, ESR, as well as the ratios of N/L and PLT/L were studied.

Results. Levels of leukocytes, neutrophils, lymphocytes, as well as N/L, WBC/MPV, MPV/L ratios were significantly higher in the patients with AMI compared to other groups. The best diagnostic value had such indicators as the total number of leukocytes (sensitivity 71.7%, specificity 69.7%, AUC 0.794), the absolute number of granulocytes (sensitivity 81.7%, specificity 77.4%, AUC 0.803), the N/L ratio (sensitivity 75.0%, specificity 71.7%, AUC 0.791) and the WBC/MPV ratio (sensitivity 76.7%, specificity 62.3%, AUC 0.760). The PLT/L ratio calculated on the 7th day of hospital stay correlated with the risk of in-hospital ($r=0.369$, $p=0.002$) and 6-month mortality ($r=0.338$, $p=0.004$) according to the GRACE score.

Conclusions. Leukocytes, granulocytes, N/L and WBC/MPV ratios had a fairly high diagnostic value for the patients with AMI. Regarding the prognostic potential assessment, only the PLT/L ratio on the 7th day of hospitalization correlated with the risk of in-hospital and 6-month mortality. This proves the importance of assessing CBC parameters not only at the time of hospitalization, but also in the dynamics of AMI.

KEYWORDS: inflammation; acute myocardial infarction; complete blood count; leukocytes.

Introduction

Inflammation plays a key role in the pathophysiology of acute myocardial infarction (AMI), both at the time of its development and later on [1, 2]. Leukocytes are the main contributors in the formation and subsequent destabilization of fibroatheroma. At the same time, they are closely related to activation of platelets increasing their prothrombotic potential [3, 4]. Different white blood cell (WBC) subtypes play their own role in this process. There are several studies that prove not only the diagnostic but also the prognostic value of assessment of leukocyte indexes and their ratios in management of the patients with AMI

[5–7]. However, most of these works are focused on the complete blood count (CBC) parameters only at the time of hospitalization. We suggest that the dynamic changes of CBC during treatment of the patients with AMI may be of value to assess the prognosis of the disease course and therefore require further study.

Methods

The study involved 152 inpatients with AMI – Group 1. Besides, two more study groups were formed: a comparison group, which included patients with stable ischemic heart disease (SIHD) (n=30) – Group 2, and a control group of healthy volunteers (n=28) – Group 3. The study was carried out at Ternopil City Municipal Hospital No. 2 (Ukraine) in October 2018 – June 2019.

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The diagnosis of AMI was verified following the clinical guidelines [8, 9]. Clinical symptoms of AMI, in particular the duration of angina pectoris and symptom-to-balloon time, the results of biochemical blood tests, angiographic protocols were evaluated. In addition, the risk of complications for acute coronary syndrome was assessed using the GRACE score [10, 11].

Blood sampling was performed from the ulnar vein in the first hour and on the 7th day after hospitalization. CBC was performed using an automated hematology analyzer; in addition, a manual count of leukocyte fractions in peripheral blood was performed, as well as the erythrocyte sedimentation rate measurement (ESR).

Statistical data was processed using the application package SPSS v25.0, as well as Microsoft Excel spreadsheets. The data were presented in the format of "mean ± standard error of the mean". The significance of the differences between the two independent samples in the case of normal data distribution was determined using the Student's t-test; in the case of abnormal data distribution – the non-parametric Mann-Whitney test. To compare three or more independent samples, one-factor analysis of variances was used – the ANOVA (analysis of variance), in the case of abnormal data distribution – the Kruskal-Wallis ranking criterion.

The ROC analysis was used to identify the diagnostic value of individual ZAK indicators. Such parameters as an area under the curve (AUC), as well as the sensitivity and specificity of the test, were analyzed.

The Pearson's correlation analysis was used to assess the relationships between the studied indicators with a normal distribution of data, and the Spearman's correlation analysis – in the case of abnormal distribution. The null hypothesis was denied at $p < 0.05$. The correlation coefficient was evaluated according to the following criteria: $r < 0.3$ – weak relationship; $r = 0.30 - 0.49$ – moderate; $r = 0.50 - 0.69$ – significant; $r = 0.70 - 0.89$ – strong; $r > 0.90$ – very strong, close to the functional association.

Results

A mean age of the patients with AMI was (62.91 ± 10.90) years old, among them the male predominated ($n = 116, 76.3\%$). More than half of the subjects had concomitant pathology in the form of hypertension ($n = 134, 88.16\%$) and diabetes mellitus ($n = 29, 19.08\%$).

Comparing the parameters of the CBC between the three groups (Table 1) it was established that in the group of patients with AMI the level of leukocytes was significantly higher than in the control and comparison groups. There was no significant difference in

Table 1. Comparison of CBC parameters between the patients with AMI, SIHD and healthy volunteers

	Group 1 (n=152)	Group 2 (n=30)	Group 3 (n=24)	P-value (ANOVA)	P value
Age, years	63.11±0.89	56.13±1.66	45.75±3.30	<0.001	0.01 ^{a,b,c}
Men, n (%)	116 (76.3)	24 (77.4)	17 (70.8)	NS	NS
WBC, 10 ⁹ /L	9.10±0.24	6.89±0.26	6.45±0.35	<0.001	<0.001 ^{a,b}
Lymphocytes, 10 ⁹ /L	1.85±0.13	2.14±0.12	2.40±0.18	0.038	0.023 ^b
Monocytes, 10 ⁹ /L	0.51±0.06	0.64±0.16	0.78±0.20	NS	NS
Granulocytes, 10 ⁹ /L	7.01±0.41	5.29±0.94	3.66±0.33	0.001	<0.001 ^b
N/L ratio	5.54±0.64	2.53±0.31	1.64±0.17	<0.001	<0.001 ^{a,b} 0.015 ^c
WBC/MPV ratio	1.03±0.05	0.76±0.03	0.78±0.04	<0.001	<0.001 ^a 0.002 ^b
MPV/L ratio	7.00±0.66	4.63±0.36	5.13±0.31	0.001	0.002 ^a <0.001 ^b
PLT/L ratio	161.59±11.86	113.99±9.07	114.36±7.83	0.004	0.02 ^a 0.001 ^b
ESR, mm/hour	11.20±0.75	8.26±0.81	7.71±0.92	0.048	<0.05 ^{a,b}

Notes:

a – comparing groups 1 and 2, *b* – comparing groups 1 and 3, *c* – comparing groups 2 and 3.

NS – not significant

WBC – white blood cells, N/L ratio – neutrophils to lymphocytes ratio, WBC/MPV ratio – white blood cells to mean platelet volume ratio, MPV/L ratio – mean platelet volume to lymphocyte ratio, PLT/L ratio – platelet count to lymphocytes ratio, ESR – erythrocyte sedimentation rate.

the absolute number of lymphocytes and granulocytes between the AMI and comparison groups, but such differences were found when comparing the AMI patients and healthy volunteers: in the AMI group, there was a significantly lower lymphocyte count and higher granulocytes. The N/L ratio differed between the three groups and was the highest in the patients with AMI ($p < 0.001$).

The ratios that simultaneously reflected two links in the pathogenesis of coronary heart disease: inflammation and platelet activation, such as PLT/L, WBC/MPV and MPV/L, were of particular interest. All of them were significantly higher in the patients with AMI, compared with the control and comparison groups.

Taking into account the obtained results of average values comparison, the diagnostic value was determined, as well as the sensitivity and specificity of certain parameters of the CBC in the patients with AMI (Fig. 1).

As seen in Table 2, the indicators such as total leukocyte count, absolute granulocyte count, N/L ratio, and WBC/MPV ratio were of the best diagnostic value.

A negative correlation was found between symptom-to-balloon time and the absolute level of lymphocytes ($r = -0.38$; $p = 0.008$), while

the positive correlation was determined with the following indicators: the N/L ($r = 0.370$; $p = 0.07$), PLT/L ($r = 0.380$; $p = 0.06$) and MPV/L ($r = 0.351$; $p = 0.011$) ratios. Also relationships were found between the duration of angina, i.e. the duration of ischemia and the number of granulocytes ($r = 0.366$; $p = 0.004$), the N/L ($r = 0.370$; $p = 0.004$) and PLT/L ($r = 0.260$; $p = 0.045$) ratios and the number of lymphocytes ($r = -0.268$; $p = 0.038$).

The relationship between the level of CPK MB and the N/L ratio ($r = 0.567$; $p < 0.001$), as well as hematocrit ($r = 0.406$; $p = 0.004$) was also established.

Any significant relationships between CBC and prognostic markers of AMI, such as the GRACE score, have not been established at this stage.

The dynamics of general blood test in the patients with AMI were further analyzed (Table 3).

A decrease in the levels of leukocytes and neutrophils was evidenced ($p < 0.001$ and $p = 0.012$). Instead, the ESR increased significantly.

Discussion

High levels of neutrophils in the patients with AMI found in our study are associated with

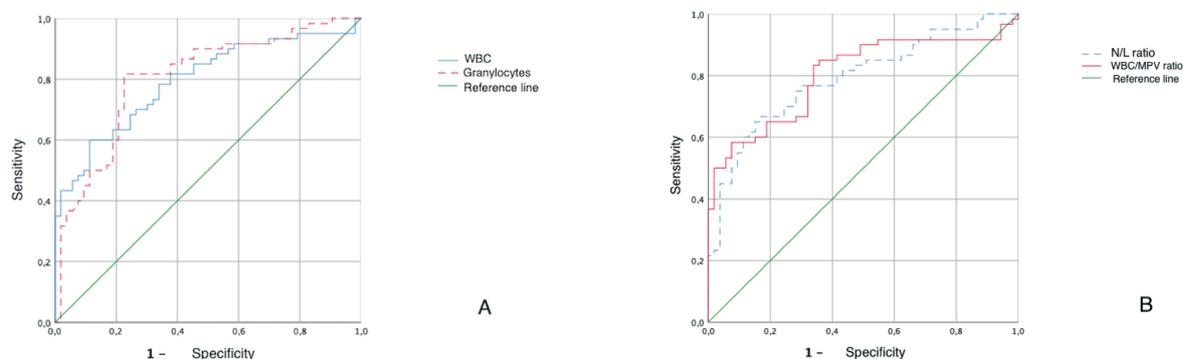


Fig. 1. Receiver operating characteristics analysis of leukocyte parameters in the AMI patients.

Table 2. Diagnostic sensitivity and specificity of the CBC parameters in the AMI patients

	AUC	Sensitivity, %	Specificity, %	Cut-off point
WBC, $10^9/L$	0.794	71.7	69.7	7.61
Granulocytes, $10^9/L$	0.803	81.7	77.4	4.99
N/L ratio	0.791	75.0	71.7	2.40
Plt/L ratio	0.654	65.0	52.8	108.49
WBC/MPV ratio	0.760	76.7	62.3	0.85
MPV/L ratio	0.051	66.7	64.2	4.44

Notes: AUC – area under the curve. WBC – white blood cells, N/L ratio – neutrophils to lymphocytes ratio, WBC/MPV ratio – white blood cells to mean platelet volume ratio, MPV/L ratio – mean platelet volume to lymphocyte ratio, PLT/L ratio – platelet count to lymphocytes ratio, ESR – erythrocyte sedimentation rate.

Table 3. Comparison of CBC parameters in the AMI patients at the time of hospitalization and on the 7th day after MI

	Day 1	Day 7	P value
WBC, 10 ⁹ /L	9.34±0.32	8.07±0.27	<0.001
Lymphocytes, 10 ⁹ /L	2.10±0.19	2.09±0.17	NS
Monocytes, 10 ⁹ /L	0.57±0.11	0.71±0.17	NS
Granulocytes, 10 ⁹ /L	6.74±0.50	5.30±0.49	0.012
N/L ratio	4.28±0.63	2.92±0.29	NS
WBC/MPV ratio	1.06±0.8	0.88±0.05	NS
MPV/L ratio	7.16±1.02	4.58±0.28	0.015
Plt/L ratio	141.45±14.50	145.31±12.05	NS
ESR, mm/hour	12.34±1.21	22.88±1.45	<0.001

Notes. WBC – white blood cells, N/L ratio – neutrophils to lymphocytes ratio, WBC/MPV ratio – white blood cells to mean platelet volume ratio, MPV/L ratio – mean platelet volume to lymphocyte ratio, PLT/L ratio – platelet count to lymphocytes ratio, ESR – erythrocyte sedimentation rate.

the formation of platelet-leukocyte aggregates in the lumen of the vessel that leads to their increase in the area of myocardial infarction. Also, neutrophils can affect platelet function by direct adhesion or by secretory factors. In contrast, the number of lymphocytes in AMI tends to decrease. We were able to confirm this pattern when comparing patients with AMI and healthy volunteers, at the same time there were no differences in the group of patients with stable coronary heart disease. This can be explained by the fact that the decrease in the number of lymphocytes is associated with physiological stress, which leads to increased cortisol levels and activates the process of apoptosis in the lymphocytes [12]. Thus, in the case of early admission to the hospital, this index may simply not have time to decrease. This is confirmed by the correlation we found, according to which the longer the angina pectoris lasted, the lower the patient's lymphocyte level was.

Given that neutrophils and lymphocytes are the cells with the opposite effect in the context of vascular inflammation, it is important to assess not only their absolute numbers but also the balance between them. One of the potential ways to do this is to evaluate the N/L ratio, which is considered to be an indicator of systemic inflammation [13]. According to our results, it not only significantly differed in the patients from the study and comparison groups, but also proved a significant positive correlation with the marker of myocardial necrosis – CPK MB.

When assessing the hemogram on the 7th day, a significant decrease in the level of leukocytes, due to neutrophils, and an increase in ESR were noted. This phenomenon is also

known as the “scissors symptom” or “crossover symptom” and is an evidence of the necroresorptive syndrome, denoted by a systemic inflammatory response to the entry of myocardial breakdown products into the bloodstream. Thus, the number of neutrophils, which is traditionally the highest on the 1st-3rd day after the development of AMI, detts to norm on the 5th-7th days due to active phagocytosis of dead neutrophils in the infarct area provided by macrophages [10]. The increase of ESR is caused by the fact that normally the charge of the erythrocyte membrane is negative and this allows them to repel each other. At the same time, pro-inflammatory proteins, in particular fibrinogen and C-reactive protein, have a positive charge and can significantly affect the state of the erythrocyte membrane causing their further aggregation [11].

When assessing the prognostic potential of individual indicators of CBC, we were able to identify it only in relation to the PLT/L ratio on the 7th day of AMI. The fact that the PLT/L ratio correlated with the risk of mortality only in 1 week is most likely explained by the platelet resistance to antiplatelet therapy during the treatment course, as well as to the stimulating effect of inflammation on megakaryocyte proliferation [14, 15]. The advantage of PLT/L ratio, compared to individual leukocyte and platelet indices, is that it reflects two interdependent processes of inflammation and platelet activation and has already proven to be a good prognostic marker of AMI, in particular regarding the prediction of left ventricular (LV) thrombus, remodeling of LV in the post-infarction period, as well as all-case mortality [14, 16–18].

Conclusions

Levels of leukocytes, granulocytes, lymphocytes, as well as N/L, WBC/MPV ratios, are available and informative markers that provide additional information about the processes of inflammation during the acute phase of myocardial infarction.

We were unable to establish a link between CBC indexes at the time of hospitalization and prognostic factors of AMI. Instead, the parameters that were obtained on the 7th day of treatment, in particular the PLT/L ratio was associated with the risk of in-hospital and 6-month mortality. Thus, we emphasize the

importance of assessing the CBC indexes and ratios in the patients with AMI in the dynamics as an inexpensive and informative method for Post-MI risk stratification.

Conflicts of Interest

Authors declare no conflict of interest.

Author's Contributions

Diana V. Zhehestovska – investigation, conceptualization, data curation, formal analysis, writing – original draft.

Marian V. Hrebenyk – data curation, writing – reviewing and editing.

ДІАГНОСТИЧНІ МОЖЛИВОСТІ ЛЕЙКОЦИТАРНИХ ПОКАЗНИКІВ СЕРЕД ПАЦІЄНТІВ З ГОСТРИМ ІНФАРКТОМ МІОКАРДА

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ТЕРНОПІЛЬСЬКИЙ ДЕРЖАВНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО,
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Вступ. Запалення відіграє визначальну роль в процесі розвитку та перебігу гострого інфаркту міокарда (ГІМ). Аналіз лейкоцитарних показників загального аналізу крові (ЗАК) є одним із способів його опосередкованої оцінки, це рутинний і доступний метод діагностики. Ми припускаємо, що динамічні зміни параметрів ЗАК в процесі лікування пацієнтів з ГІМ можуть становити цінність, зокрема і для оцінки прогнозу подальшого перебігу захворювання, а тому потребують подальшого вивчення.

Мета. Оцінити діагностичний та прогностичний потенціал лейкоцитарних індексів ЗАК, зокрема кількісних рівнів лейкоцитів (WBC), лімфоцитів (L), нейтрофілів (N) та співвідношень N/L, WBC/MPV (середнього рівня тромбоцитів, MPV), PLT/L (співвідношення числа тромбоцитів до кількості лімфоцитів) у пацієнтів з ГІМ на момент госпіталізації та на 7-му добу перебування в стаціонарі.

Матеріали та методи. В дослідженні взяло участь 204 особи (152 пацієнтів з ГІМ (Група 1), 30 пацієнтів з стабільною ІХС (Група 2) та 24 здорові добровольці (Група 3)). Досліджували показники гемограми та їх співвідношення, зокрема рівні лейкоцитів, лімфоцитів, нейтрофілів, ШОЕ, а також співвідношень N/L ratio та PLT/L ratio.

Результати. Рівні лейкоцитів, нейтрофілів, лімфоцитів, а також відношення N/L, WBC/MPV, MPV/L були достовірно вищими серед пацієнтів з ГІМ порівняно із іншими групами. Найкращою діагностичною цінністю володіли такі показники, як загальна кількість лейкоцитів (чутливість 71,7%, специфічність 69,7%, AUC 0,794), абсолютна кількість гранулоцитів (чутливість 81,7%, специфічність 77,4%, AUC 0,803), відношення N/L (чутливість 75,0%, специфічність 71,7%, AUC 0,791) та WBC/MPV (чутливість 76,7%, специфічність 62,3%, AUC 0,760). Співвідношення PLT/L виражене на 7-ий день перебування в стаціонарі корелювало з ризиком госпітальної ($r = 0,369$, $p = 0,002$) та 6-ти місячної смертності ($r = 0,338$; $p = 0,004$).

Висновки. Показники лейкоцитів, гранулоцитів та співвідношення N/L і WBC/MPV володіли доволі високою діагностичною цінністю серед пацієнтів з ГІМ. Стосовно оцінки прогностичного потенціалу, то лише відношення PLT/L на 7-ий день госпіталізації корелювало з ризиком госпітальної та 6-ти місячної смертності. Це вказує на важливість оцінки ЗАК не лише на момент госпіталізації, а й в динаміці перебігу ГІМ.

КЛЮЧОВІ СЛОВА: запалення; гострий інфаркт міокарда; загальний аналіз крові; лейкоцити.

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Received 28 Oct 2020; revised 9 Dec 2020;
accepted 14 Dec 2020.

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