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Research Article

MONOCYTES AND LYMPHOCYTES PROGNOSIS TOOLS AMONG STROKE PATIENTS

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ABSTRACT

Objectives: The values of white blood cell types were evaluated to determine which one is the most potential index in the diagnosis of patients with ischemic stroke (IS). Besides, neutrophil-lymphocyte ratio, neutrophil-to-monocyte ratio, and lymphocyte-to-monocyte ratio (LMR) were determined to confirm whether there is a relationship between these parameters and stroke.

Methods: One thousand files of patients admitted between January 2017 and March 2020 to intensive care units at Alia Governmental Hospital in Hebron-Palestine were reviewed and evaluated. Only a total of 87 patients were found to meet our inclusion criteria and included in the study and a total of 913 patients were excluded. Besides, complete blood counts of a total of 95 patients' data were also collected randomly from Private Lab in Hebron district, and were considered as healthy individuals (HI); that is, a control group. The data were introduced to the excel program 2010 version to facilitate reading and analysis. GraphPad Prism version (8.3.4.) was used to analyze the data. The data of patients and HI were compared to determine which value/s of these blood components is associated with the risk of stroke.

Results: Different types of white blood cells; monocytes, lymphocytes, and neutrophils for stroke and healthy patients were collected and compared. Besides, the platelet count was also compared. It was found a marked significant decrease in the monocytes counts in all stroke patients (100–1500 cell/ul) compare to monocytes counts in the healthy donors (310–1740 cell/ul). Lymphocytes were also significantly decreased among all stroke patients (300–8600 cells/ul) compare to HI (910–9000 cells/ul). There was no significant increase in neutrophils in stroke patients. The ratios of neutrophils to monocytes and neutrophils to lymphocytes in stroke patients were compared too. The results showed a significantly elevated neutrophil ratio compare to monocytes and lymphocytes in all stroke patients. The platelet counts in stroke patients were also found less compared to HI. The percentages of these blood components were also evaluated.

Conclusion: It is demonstrated that patients with stroke have lower lymphocyte and higher monocyte counts, and therefore, lower LMR values compared to the control group. It was found that MLR was significantly correlated with IS. LMR is associated with functional outcomes in patients with stroke.

Keywords: Lymphocyte-to-monocyte ratio, Acute ischemic stroke, Stroke severity, Prognosis, Neutrophil to monocyte.

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INTRODUCTION

Stroke is the third leading cause of death and a major cause of disability in Western countries. Ischemic stroke (IS) is the most common type of stroke and is about 80% of all strokes [1]. Inflammation is a cellular response to protect the body against harmful things, such as infections, injuries, and toxins. When something damages the cells, the body releases chemicals that trigger a response of the body's immune system. IS is one of the main causes of mortality and morbidity in the world. The occlusion of cerebral arteries in IS patients is the most common cause of emergency admission. IS accounts for 80–85% of all cerebrovascular diseases as mentioned [2]. IS releases a very complex cascade of molecular and cellular effects that are related to each other [3]. The inflammatory response is one of the major critical factors contributing to the pathophysiological process of IS. There are different inflammatory markers such as C-reactive protein (CRP) and fibrinogen used for the prognosis of IS [3]. It was indicated that elevated plasma levels of inflammatory cytokines, CRP, and chemokines are associated with future cardiovascular risk [4]. Plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin were observed to be increased both in large intracranial artery disease and small artery disease [5]. Plasma levels of ICAM-1 and monocyte chemoattractant protein-1 (MCP-1) were noted to be high in patients with IS and myocardial infarction [6,7]. Thus, it has been suggested that white blood cell subtypes can also be used as a marker of inflammation [8]. It has been indicated that lymphocyte count may decrease in IS patients after ischemia [8,9]. It is thought that the suppression of lymphocyte count can be secondary to lymphocyte apoptosis and redistribution of lymphocytes to lymphatic organs which are induced by stress after ischemia. It has also been revealed that the decrease in lymphocyte count is associated with poor prognosis after IS and gives a negative role in long-term functional recovery. It is considered that microglial cells located throughout the brain are activated rapidly after ischemia and are the major cells that contribute to inflammation after injury. However, for many years, clinical observations showed that plasma levels of inflammatory cytokines were increased after stroke onset, and immune cells, especially monocytes/macrophages and T-lymphocytes, existed in stroke lesions and related to exaggerate brain damage.

Epidemiological studies have shown that elevated leukocyte count was associated with the risk for 1st time myocardial infarction and IS [10-12] and the risk of recurrent myocardial infarction and IS in a high-risk population [13]. These observations indicate that inflammatory events that occur in stroke patients increase the risk of stroke recurrence. Studies showed that these inflammations occurred before stroke onset. Plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), sICAM-1, sE-selectin, and MCP-1 were also elevated in patients with essential hypertension in the absence of other diseases [14-17]. However, anti-inflammatory strategies were shown to suppress the incidence of stroke. Thus, it has been suggested that inflammation might be a risk factor for stroke. Therefore, it is important to find the role of inflammation, especially monocytes/macrophages, in IS. It was found

that monocytes increase in peripheral blood level after IS, then travels to the brain where it blocks a blood vessel (Embolus) and contributes to the expansion of the ischemic lesion [3,18]. Lymphocyte/monocyte ratio (LMR) is a new indicator of the inflammation response and is also found to involve in many types of cancers [19,20]. Besides, low LMR levels are found to be closely related to the progress of coronary artery disease [21]. Different studies showed low LMR levels after acute IS (AIS) [22]. It has revealed that some of the white blood cell subtypes are associated with mortality due to cerebrovascular diseases [23]. However, the correlation of LMR with mortality in patients with AIS needs further studies. Several studies investigated the relationship between peripheral leukocytes and stroke outcomes too [24-27]. However, most of such studies were focused on neutrophil or a neutrophil-lymphocyte ratio (NLR) and were not based on the concept of stroke-induced immunosuppression. Hence, we hypothesized that the LMR of peripheral blood could be an indicator to represent the change in the peripheral immune system after stroke. To confirm our hypothesis, we evaluated whether LMR and other ratios such as NLR and neutrophil-to-monocyte ratio (NMR) are associated with the risk of stroke patients with AIS. We collected complete blood counts (CBC) counts for stroke patients and control groups including monocytes, lymphocytes, neutrophils, and platelets. We have compared each of the values between the two groups. The ratios of all these components were also calculated to confirm which value/s or/and ratio/s of these blood components is associated with the risk of stroke and to confirm whether LMR and other ratios such as NLR and NMR are a significant marker with AIS. Subsequently, this study aimed to retrospectively compare the LMR values between patients with IS and control and to determine the correlation of LMR in patients with IS and/or any other components correlations. As far as we know, our study is the first in Palestine to study the association between the LMR and the outcome of AIS. The percentages of these blood components and the platelet counts in stroke patients were also evaluated. The parameters of these components; cell counts, ratios, and percentages, were evaluated and discussed in this research to determine which one of these values is the most potential index in the diagnosis of patients with IS.

METHODS

We have reviewed and evaluated 1000 files of patients admitted to intensive care units between January 2017 and March 2020 at Alia Governmental Hospital in Hebron-Palestine. Patients were included in our study if they have met all of the following inclusion criteria: (1) Lacunar stroke and AIS, (2) within 24 h from stroke onset, and (3) strokes in the anterior circulation. Patients with the following conditions were excluded: (1) Autoimmune diseases, (2) cancers, (3) hematologic disorders, (4) infectious diseases (5) taking the immunosuppressant, and (6) other cardiovascular diseases. We have reviewed patients' files after getting approval from the Palestinian Ministry of Health (MOH). The patients' identities remained confidential and the data used for research purposes only.

Only 87 patients were found to meets our inclusion criteria and included in the study and a total of 913 patients were excluded. Besides, CBC data of a total of 95 patients were also collected randomly from Al-Waed Private Lab in Hebron district, and were considered as healthy individuals (HI); that is, the control group. HIs were selected as those with a routine CBC checkup and healthy with a non-recognized disease, especially stroke or/and its related diseases or stroke consequences diseases. The data for patients and control group were introduced to the excel program 2010 version to facilitate reading and analysis. The data have been divided and separated into rows and columns according to the research requirements. GraphPad Prism version (8.3.4.) was used to analyze the data. The parameters of white blood components; cell counts, ratios, and percentages, were evaluated and discussed in this research study. Besides, the platelet counts were collected for both groups.

RESULTS

Different types of white blood cells; monocytes, lymphocytes, and neutrophils for stroke and healthy patients, were collected and compared. Furthermore, the platelet count was determined. The values of these components; cell counts, ratios, and percentages, were evaluated and discussed in this research to determine which one is the most potential index in the diagnosis of patients with IS. It was found a marked significant decrease in the monocytes counts in all stroke patients (100–1500 cell/ul) compare to monocytes counts in the healthy donors (310–1740 cell/ul). Lymphocytes were also significantly decreased among all stroke patients (300–8600 cells/ul) compare to HI (910–9000 cells/ul). There was no significant increase in neutrophils in stroke patients. The ratios of neutrophils to monocytes and neutrophils to lymphocytes in stroke patients were compared too and evaluated. The results showed a markable elevated neutrophil ratio compare to monocytes and lymphocytes in all stroke patients. The platelet counts in stroke patients were found less compared to HIs. Other ratios of blood components were also evaluated. Out of 1000 patients, 87 patients were included in this study, after the exclusion of 913 patients according to the exclusion criteria shown in the methods section. A comparison between leukocyte counts and their respective ratios was evaluated too. It was found that the Lymphocyte/monocyte ratios were lower in patients with acute Ischemic stroke (AIS); while the neutrophils' percentages to either lymphocytes or monocytes were higher in the patients' group; these were the most significant parameters in our study. However, the results divided according to the type of blood components and evaluation methods; that is, cells count, ratios, and percentages of the components as following:

CBC of monocytes and T-lymphocytes cells in stroke patient versus healthy donors

Our preliminary results showed that the monocytes in the presence of high-molecular-weight heparin have a positive impact on MMP-9 regulation [27]. Thus, it was reasonable to investigate the role of monocytes in stroke patients compared to HI. The initial result of this study showed that there was a markable significant decrease in the monocytes counts in all stroke patients (100–1500 cell/ul) compare to the monocytes counts in the healthy donors (310–1740 cell/ul), as shown in Fig. 1. Thus, this supports the assumption that monocytes appear to be the major key player in the pathophysiology of stroke. To investigate the other immune cell types which might also have an impact on stroke, we determined the cell counts of lymphocytes and neutrophils on the presence or absence of stroke. As expected, we could reveal that in the presence of stroke, lymphocytes are significantly decreased, as shown in Fig. 2. This is further confirmation that other immune cell types have a role in stroke. The decrease of lymphocytes is considered a significant decrease among all stroke patients in which it varies between 300 and 8600 cell/ul in stroke patients and between 910 and 9000 cell/ul in HIs, as shown in Figs. 1 and 2. When the cell count of neutrophils of stroke patients and healthy individuals were compared; it was noticed that the

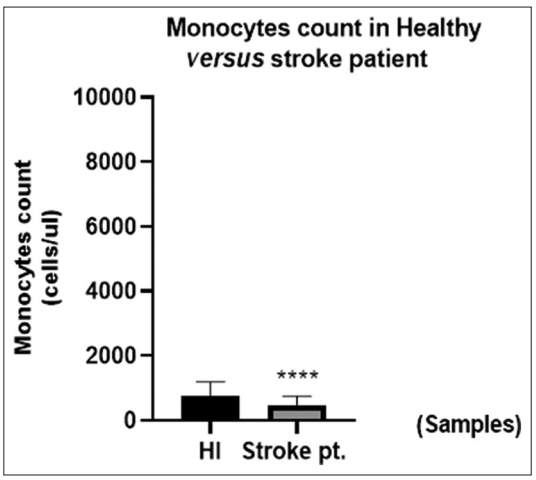

Fig. 1: Significant reduction of monocytes whole blood samples of stroke patients. The cell count of monocytes in the corresponding patients was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001

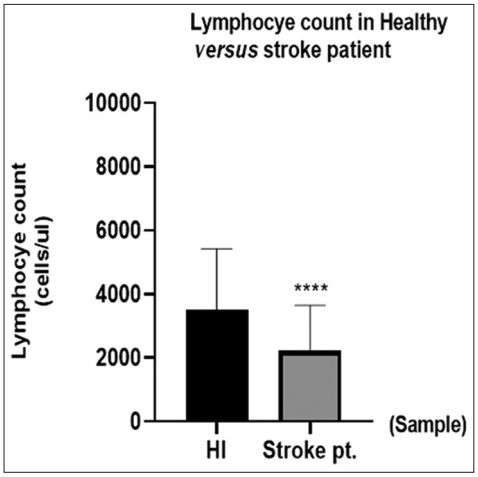

Fig. 2: Significant reduction of lymphocytes in whole blood samples of stroke patients. The cell count of lymphocytes in the corresponding patients was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001

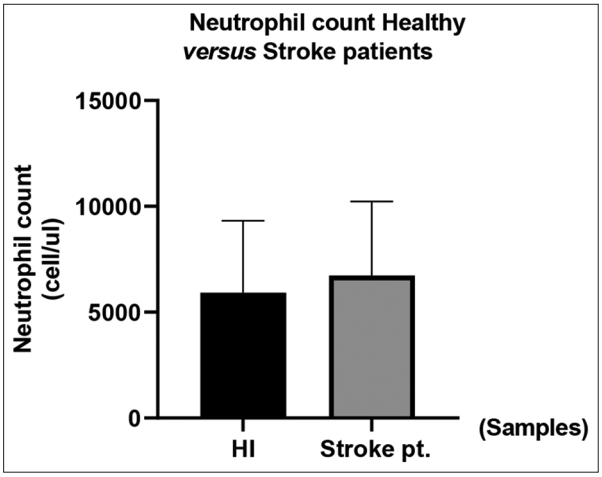

Fig. 3: Non-significant increase of neutrophils in whole blood samples of stroke patients. The cell count of neutrophils in the corresponding patients was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.1171

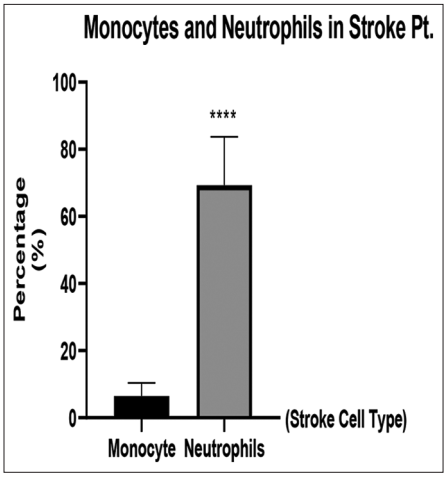

Fig. 4: Comparison between monocytes and neutrophils ratios among stroke. Both cell ratios in the corresponding patients were compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001. F-test was used to compare variances in which results were considered significant at p≤0.0001

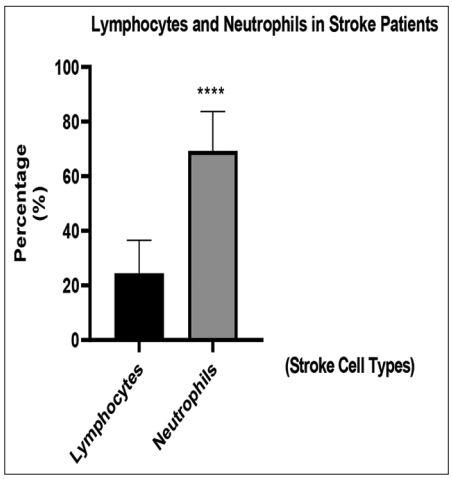

Fig. 5: Comparison between lymphocytes and neutrophils ratios among stroke. Both cell ratios in the corresponding patients were compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001. F-test was used to compare variances in which results were considered non-significant at p≤0.1113

cell count of neutrophils in the blood samples of the patients is slightly increased compared to the healthy group, as shown in Fig. 3, thus there was no significant difference in neutrophils between the two groups.

Comparing the monocytes and lymphocytes in the HIs with the normal range values; it is shown that once the person is in a healthy state, the count of the monocytes is within the normal range, which is between 200 and 950 cell/ul, and the lymphocytes count is also in the normal state, which is between 850 and 3500 cell/ul, as shown in Figs. 1 and 2. Thus, an adverse effect on these two cell counts happened during the stroke, this clarifies that there is a relationship between both cell types themselves and the stroke. However, we also compared the cell counts between both neutrophils/monocytes and neutrophils/T-lymphocytes to confirm if there are any relationships between these components in stroke patients or to exclude the neutrophils. Thecomparison results between both cell types monocytes/neutrophils and lymphocytes/ neutrophils ratios are summarized in Figs. 4 and 5. It was found that the neutrophils ratios were markedly elevated in all stroke patients compared to monocytes (Fig. 4) and lymphocytes (Fig. 5). As expected, we could not reveal any significant effects after comparing the neutrophils/monocytes cell counts ratio (Fig. 6) or neutrophils and T-lymphocytes cell counts ratio (Fig. 7), this supports the assumption that; in the pathophysiology of stroke, the monocytes is a key player in addition to its connection to lymphocytes. The cell count of platelets in the blood samples of the stroke patients is slightly decreased compared to the healthy group, as shown in Fig. 8, thus no significant difference in platelet between the two groups. However, surprisingly on further investigation, there were new promising results concerning the ratio of platelets and neutrophils counts. As the results of the neutrophils did not have any regulatory effect on monocytes or lymphocytes, while it has significant effects in the platelet (data not shown); more studies will be carried out in platelets to verify this.

Monocytes, lymphocytes, and neutrophil ratio as a predictor tool in stroke

Since there is an interaction between monocytes and other immune cells further analysis was carried out to reveal if the immune cell counts could be a better diagnostic or prognostic approach or whether certain

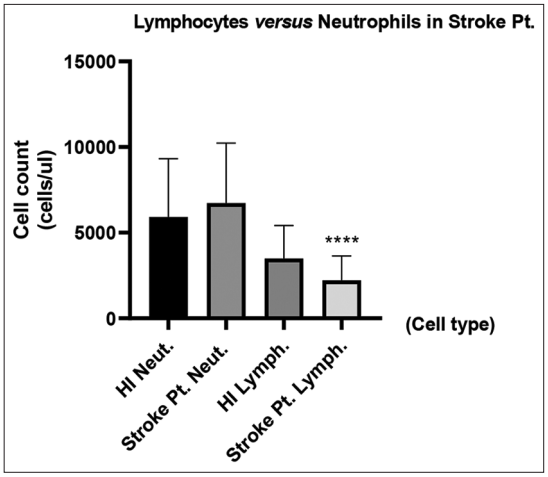

Fig. 6: Lymphocytes versus neutrophils in stroke patients. Cell counts for the corresponding stroke patients were compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001. F-test was used to compare variances in which results were considered significant at p≤0.0078

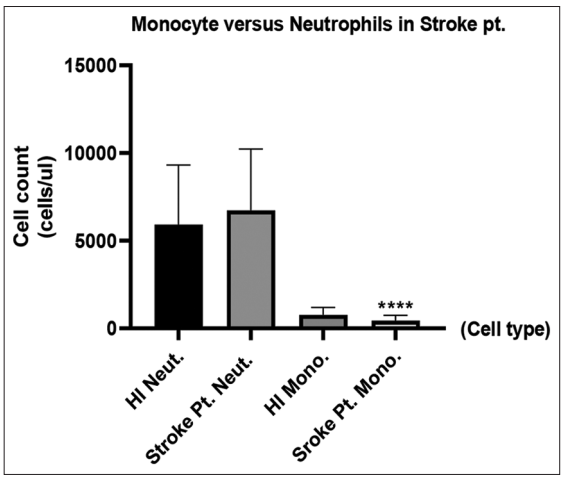

Fig. 7: Monocytes versus neutrophils in stroke patients. Cell counts for the corresponding stroke patients were compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001. F-test was used to compare variances in which results were considered significant at p≤0.0010

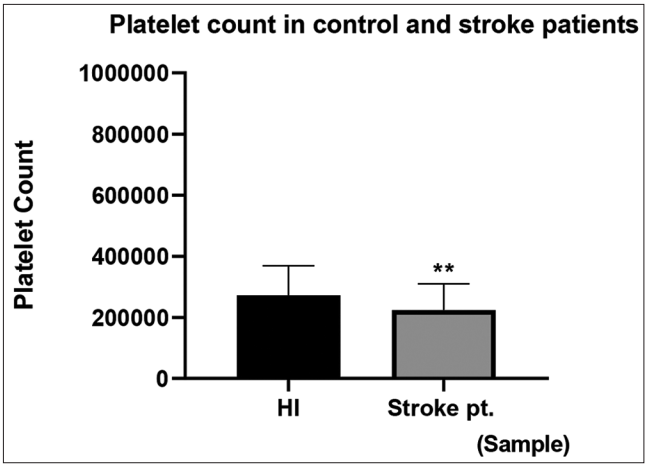

Fig. 8: Platelets count in stroke patients. Cell counts for the corresponding stroke patients were compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0011. F-test was used to compare variances in which results were considered non-significant at p≤0.3034

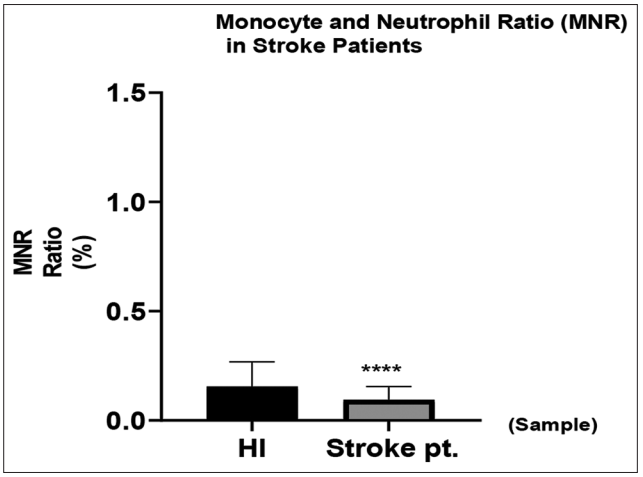

Fig. 9: Monocyte/neutrophils ratio a negative prediction tool among stroke patients. The ratio of the corresponding studied groups was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001. F-test was used to compare variances in which results were considered significant at p≤0.0001. Mann–Whitney U-test was significant at p≤0.0001

immune cells could be considered as a better predictive approach. Thus, the ratio between monocytes and neutrophils in HIs and stroke patients was calculated and found that there is a slight significant decrease between monocytes/neutrophils ratio in stroke patients in contrast to HI at p≤0.0001, respectively. The ratio of monocytes/neutrophils was between 0.01–0.2% in stroke patients and 0.01–0.5% in HIs, as shown in Fig. 9. When the ratio between monocytes/lymphocytes was determined; a massive significant increase in all stroke patients 0.15–0.8% and 0.09–0.5% in HIs existed, as shown in Fig. 10. Moreover, to support our results, the ratio of lymphocytes/monocytes (Fig. 11) or lymphocytes/neutrophils (Fig. 12) was also calculated for both studied groups. The results showed that there is a decrease in the ratios of both lymphocytes/monocytes (0.3–25%) or lymphocytes/neutrophils (0.03–1.2%), which was considered a significant decrease in lymphocytes/neutrophils but a non-significant decrease in lymphocytes/monocytes.

To elucidate the role of neutrophils if they could be a better predictive tool of stroke, neutrophils-monocytes percentage ratio (NMR) was calculated, as shown in Fig. 13. It revealed that neutrophils have a role in the prediction of stroke similar to monocytes and could be used as s supportive predictor, as the neutrophils/monocytes ratio was significantly increased (Fig. 13) in contrast to neutrophils/lymphocytes (Fig. 14). This shall support our assumption that monocytes are the key players in stroke disease. Thus, the predictive tool of stroke is dependent initially on monocytes cell counts, and the second parameter is lymphocytes, which show a kind of adverse effect. As previously mentioned and shown in Figs. 6 and 7, neutrophil cell counts are increased non-significantly in stroke patients in contrast to monocytes cell count which shows a massive significant increase in all stroke patients. Interestingly, we could reveal a significant reduction in the neutrophils/lymphocytes/platelet ratio (NLPR) in stroke patients (Fig. 15).

Investigating the prevalence of monocytes ratio among stroke and HI showed that the majority of stroke patients had a monocytes ratio between 5.1–10% and this represented 37.2% of the total stroke

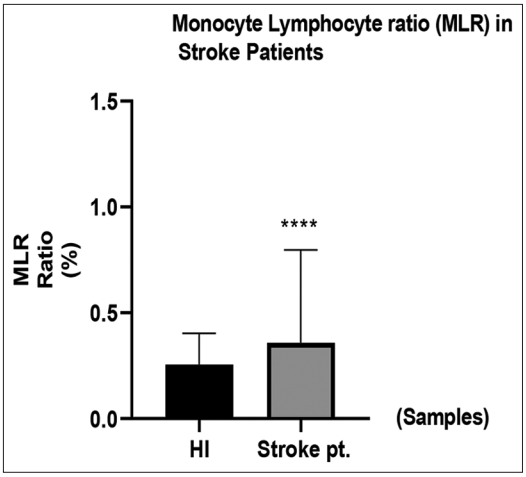

Fig. 10: Monocyte/lymphocytes ratio a positive prediction tool among stroke patients. The ratio of the corresponding studied groups was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001. F-test was used to compare variances in which results were considered significant at p≤0.0001. Mann–Whitney U-test was considered non-significant at p≤0.4385

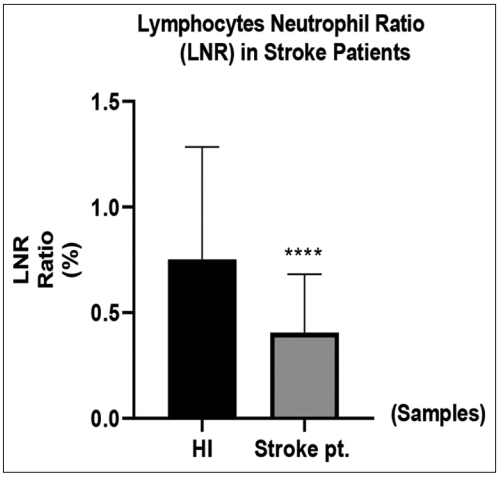

Fig. 12: Lymphocyte-neutrophil ratio a negative prediction tool among stroke patients. The ratio of the corresponding studied groups was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.0001. F-test was used to compare variances in which results were considered non-significant at p≤0.0001. Mann–Whitney U-test was significant at p≤0.0001

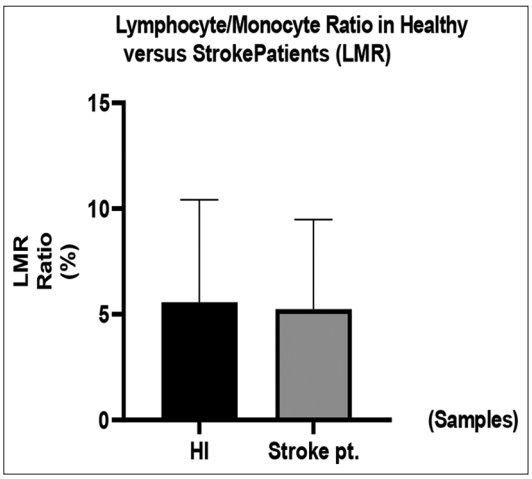

Fig. 11: Lymphocyte-monocyte ratio a non-useful prediction tool among stroke patients. The ratio of the corresponding studied groups was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0.6463. F-test was used to compare variances in which results were considered non-significant at p≤0.2194. Mann–Whitney U-test was non-significant at p≤0.5097

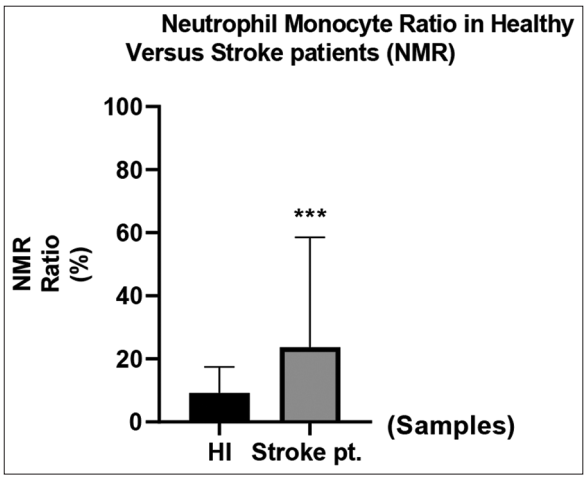

Fig. 13: Neutrophil-monocyte ratio a positive useful prediction tool among stroke patients. The ratio of the corresponding studied groups was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0006. F-test was used to compare variances in which results were considered non-significant at p≤0.0001. Mann–Whitney U-test was significant at p≤0.0001

patients' profiles (32 patients), as shown in Table 1. The range between 0.1 and 5% represented 34.8% of the stroke patient profiles (30 patients) which is the second common monocytes ratio (Table 1), 10.1–15% is 16% of the stroke patient profiles (Table 1). The ratio of monocytes between 15.1–20% was the least common one in stroke patients (Table 1). Among HIs, a monocytes ratio between 0.1 and 5% was the most common ratio which is in 98% of HI; this ratio is the normal ratio of monocytes in their CBC (Table 1).

Table 2 shows the lymphocytes ratios; as the ratio between 30.1 and 35 was the most common ratio interval in which 17.2%, 15 patients of the total stroke CBC reports, the ratio between 10.1 and 15 was the second group ratio interval with 14.9%. Lymphocytes ratio among stroke patients with the ratios interval 21.1–25, and 25.1–30, was the third distributed intervals with a total of 12.6% of the total stroke patients' data. Concerning the lymphocytes ratio in HIs, as represented in Table 2, the majority of the HIs had a lymphocytes ratio in the interval between 0.1 and 5 which represented 84.21% of the total HIs CBC reports. In addition, the interval ratio between 5.1 and 10 was only in 15.79% of CBC reports of the total HI.

DISCUSSION

Stroke is a major cause of morbidity and mortality worldwide. Stroke is a leading problem worldwide and Palestine has high cases of mortality due to the stroke and its complications such as a sudden heart attack, blood clot, thrombus formation, and embolus formation. A stroke can sometimes cause temporary or permanent disabilities, depending on how long the brain lacks blood flow and which part was affected. Complications may be neurological and psychological, which include paralysis or loss of muscle movement, difficulty talking or swallowing, memory loss or thinking difficulties, emotional problems, pain, changes

Table 1: Prevalence of monocytes ratio among stroke and healthy donors

%	(0.1–5)	(5.1–10)	(10.1–15)	(15.1–20)	(21.1–25)	(25.1–30)	(30.1–35)	(35.1–40)	(40.1–45)	(45.1–50)	(51–55)	(55.1–60)
No. of patients and monocytes ratio in stroke	30 (34.8%)	32 (37.2%)	14 (16%)	2 (2.32%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
No. of patients and monocytes ratio in healthy individuals	94 (98.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

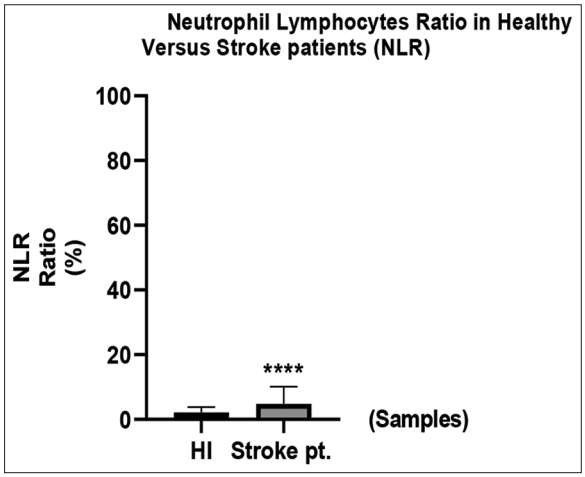

Fig. 14: Neutrophil-lymphocyte ratio a positive useful prediction tool among stroke patients. The ratio of the corresponding studied groups was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0001. F-test was used to compare variances in which results were considered non-significant at p≤0.0001. Mann–Whitney U-test was significant at p≤0.0001

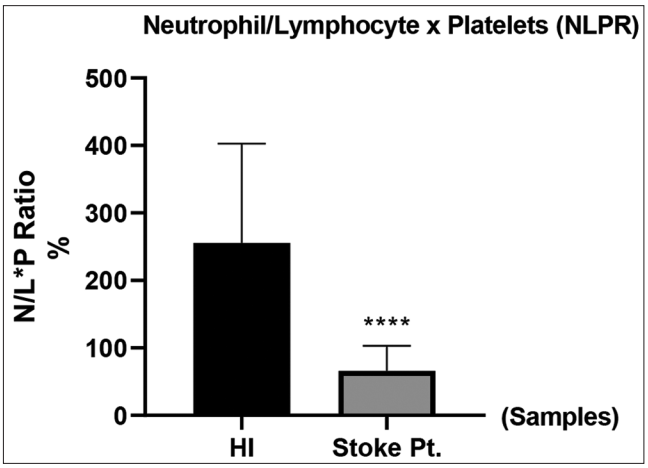

Fig. 15: Neutrophil/lymphocyte x platelets ratio a useful prediction tool among stroke patients. The ratio of the corresponding studied groups was compared by the unpaired t-test with Welch correction. Results were considered significant at p≤0001. F-test was used to compare variances in which results were considered non-significant at p≤0.0001. Mann–Whitney U-test was significant at p≤0.0001

in behavior, and self-care ability [28-30]. In a healthy state, the body's immune cells are always the first line of defense in the era of stroke. The immune system is involved in all stages of stroke, from the pathogenesis of risk factors to neurotoxicity, to tissue remodeling and repair. The IS starts in the blood vessels, where arterial occlusion results in hypoxia, reactive oxidative species production, and changes in shear stress across the lumen wall. In clinical practice, high-sensitivity CRP and erythrocyte sedimentation rate (ESR) are the most widely used inflammatory markers. The NLR, neutrophil-to-monocyte (NMR), LMR, and platelet-to-lymphocyte ratio (PLR) are reported to be inflammatory biomarkers in stroke patients [31,32].

The pathophysiology of stroke is complex and involves numerous processes, including energy failure, loss of cellular ion homeostasis, acidosis, increased intracellular calcium levels, excitotoxicity, free radical-mediated toxicity, generation of arachidonic acid products, cytokine-mediated cytotoxicity, complement activation, disruption of the blood–brain barrier (BBB), activation of glial cells, and infiltration of leukocytes. However, different immune cell interacts together to fight such a battle, whether it is due to genetics, risks factors, coagulation state, or any other complication or disease that can lead to stroke and its consequence complications [33]. The preliminary work of our group studied the impact of different anticoagulants on the regulation of matrix metalloproteinase 9, the cellular and molecular effect of these anticoagulants on the regulation of MMP-9. It was initially induced from the monocytes in *in vitro* experiments, which performed on different cell lines as THP-1; which mimics the monocytes, and Jurkat cell which mimics T-lymphocytes in humans. The preliminary finding showed that there was an interaction between both cells and they are dependent on the other [27]. This gave us attention to investigate the role of immune cells including monocytes in stroke patients compared to HI. The calculation of immune cell ratios is considered to be simple, cheap, and non-invasive as a tool for the diagnostic or prognostic markers in stroke, thus investigating the immune cell distribution could presumably provide insights into the disease progression. Therefore, to extend our preliminary work, we studied the effect of such immune cells *in vivo*, concentrating mainly on monocytes and lymphocytes. Therefore, we focused on this research study in the analysis of the CBC reports for stroke patients and compared them with healthy donors. According to our knowledge, this study so far is the first one in Palestine, which studies the relationship of the different components of the white cell in stroke patients. It was found that there was a significant reduction in monocyte cell counts in all stroke patients group, and this reduction is always supported by a marked significant increase in the lymphocytes counts. From our point of view, it seems that the major monocytes which already distributed in the bloodstream; once there is a stroke, these monocytes travel from the bloodstream into specific macrophages either M1 or M2, this explained why there was a significant decrease in the monocytes cell counts in the bloodstream of all stroke patients. In our research study, we did not investigate which type of monocytes involved in the stroke; whether it is the classical or the non-classical forms. Therefore, it is conceivable to speculate the type of monocytes, as some studies had found that in the stroke, some kind of monocytes

Table 2: Prevalence of lymphocytes ratio among stroke and healthy donors

%	(0.1–5)	(5.1–10)	(10.1–15)	(15.1–20)	(21.1–25)	(25.1–30)	(30.1–35)	(35.1–40)	(40.1–45)	(45.1–50)	(51–55)	(55.1–60)
No. of patients and lymphocytes ratio ranges in stroke	2 (2.29%)	10 (11.4%)	13 (14.9%)	8 (9.1%)	11 (12.6%)	11 (12.6%)	15 (17.2%)	9 (10.3%)	5 (5.74%)	2 (2.29%)	1 (1.1%)	0 (0%)
No. of patients and lymphocytes ratio ranges in healthy individuals	80 (84.21%)	15 (15.79%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

is increased [33], while other studies reported that the increase of monocytes level during stroke could be due to the presence of a stroke associated infection or to severe strokes [34].

The platelet cells count from the CBC reports of both groups was determined and compared. Interestingly, the platelet cell counts were significantly decreased among stroke patients, although it is still considered within the normal range. In all the stroke patients, the decrease was more toward the lower limit of the normal range, in contrast to HI as their platelet cell counts were within different ranges of the normal range. Since the platelets are among the major component of blood and responsible to prevent any hemorrhage, the platelets count in stroke patients was found to be more toward the lower limit of the normal range as mentioned; this further indicates the risk for thrombocytopenia (i.e. low platelets counts) state or due to the presence of pseudo-transient thrombocytopenia. This could be an actual indicator for the prediction of the stroke, but further research studies should be carried out to confirm this finding; due to the less knowledge about the proper ways of blood sample collection of stroke patients, since most of the stroke samples are collected in EDTA tubes, which is considered an *in vitro* error and could impact the count of platelets in the sample [35]. Therefore, we may recommend some further studies to be conducted using a different type of anticoagulant for stroke patients, since in the previous studies of our group, we showed that pre-analytics play a key role in the process of determination of the MMP-9 concentration and it could have similar effects in the analysis of stroke patients blood samples [36]. Furthermore, it is further recommended to measure the count of platelets before any management or treatment thrombolysis of stroke patients. According to our knowledge, this is the first study that checks the role of different parameters in the inflammations specifically the stroke and could be used as a tool in the prediction and progress of stroke. On the other hand and interestingly, our results showed that among the corresponding patients; the lymphocytes were elevated in comparison to monocytes in stroke patients, but were significantly lower in contrast to the lymphocytes counts in the healthy group. Our finding was consistent with another finding which showed that the presence of stroke may induce a dramatic loss of lymphocytes especially the T-lymphocytes [37], furthermore, the post-stroke induces also a further loss of T-lymphocytes, this due to the immune depression fact [38]. For the 1st time, we showed that another immune cell (i.e. neutrophils) was slightly elevated among stroke patients, although this elevation is considered a non-significant elevation, still, there was an increase in comparison to the HIs, this might contribute to the fact that within the minutes of the stroke occurrence, the first line of the immune cells is the neutrophils, which are recruited to the brain, therefore, it is predictable to find a transient increase in neutrophils cell counts [39], but this cell count non-significantly increased.

Concerning our study finding, MNR was shown to be a kind of negative prediction tool among stroke patients since this ratio between both monocytes and neutrophil cell types was significantly decreased in contrast to the same cell types in HIs. Moreover, we showed that MLR is markedly and significantly elevated in all stroke patients, this indicated that among the pathophysiology of stroke; both cell types (monocytes and lymphocytes) have an association between them, which is shown to be a kind of positive prediction tools among stroke patients. A retrospective study has shown that MLR is associated with carotenoid stenosis in ischemic patients [40]. Unexpectedly, LMR was a non-useful prediction tool among our studied group, in which there was no significant effect between both cell ratios, conversely, some studies showed that LMR was an independent factor in the severity of the stroke and its consequences [38]. Among our results, LNR showed a negative prediction tool among stroke patients, therefore, this indicates that among these; there is an association between these cell types and the stroke. Surprisingly, we revealed that the NMR was a positive useful prediction tool among these stroke patients. In which it was markedly increased with about 2–3-fold induction; therefore, this supports our assumption that the monocytes are a key player in the stroke. Besides, the NLR is found to be a positive useful prediction tool among stroke

patients. Our finding was consistent with pilot studies that determined the neutrophils-to-lymphocytes and lymphocytes-to-monocytes ratios, which showed that the stroke patients with higher NLR are expected to have a more severe stroke [41] and the association of NLR and MNR can predict the functional outcome of stroke after mechanical thrombectomy [42]. From our study, we revealed a new promising tool that is based on measuring the NLPR. Presumably, this approach could be a better tool for diagnosis, mono, and prognosis of stroke; since our study showed that there was a marked significant decrease in the NLPR; once we divided the total count of neutrophils on the lymphocytes times platelets count. This predictor is most likely to be a novel prognosis tool in stroke patients. Our study confirmed that some tools could be potentially used as positive predictor tools among stroke; in which they are increased in addition to others. Besides, others could be used as a kind of negative prediction tool; in which they decreased in stroke patients. Therefore, these kinds of markers are impressive and can be added to the panel of stroke markers or inflammatory research; in addition to its correlation with immune cells. Thus, the utility of these could provide informative knowledge to the profiles of patients with stroke. Further studies that focus on the type of different kinds of monocytes, lymphocytes, and neutrophils are still needed to confirm if these tools contribute as the same prediction effect among them. Besides, our research study has some limitations. First, this study is a retrospective single-site study, a prospective, and multisite cross-sectional research should be carried out. Second, the relationship between MLR and the incidence of major adverse cardiac and cerebral vascular events and death was not explored in this study. Thus, further study is needed to be performed in the future.

CONCLUSION

The immune system has a key role in brain injury. A better understanding of the interactions between the immune system and the brain can aid physicians who care for patients with stroke and other forms of central nervous system injury. The main finding of this research study was that NLR in stroke patients was markedly high. Therefore, patients with higher NLR may be expected to have a more severe stroke. The monocytes/lymphocytes percentage ratio is also an independent risk factor of stroke. It is demonstrated that patients with stroke have lower lymphocytes and higher monocyte counts, and therefore, lower LMR values compared to the control group. Our study found that MLR was significantly correlated with IS and LMR is associated with functional outcomes in patients with stroke. Therefore, MLR might be considered a potential index in the diagnosis of patients with IS. The link between stroke severity and NLR and MLR deserves further study with large sample size.

ETHICAL CONSIDERATIONS

The study was approved by the Palestinian MOH. The identities of patients remained unknown and confidential; the data only used for research purposes. The consent of the individuals to participate in the survey and to collect and process their data was obtained from all participants. All the participants in the research study could withdraw at any time.

CONFLICTS OF INTEREST AND FINANCIAL DISCLOSURE

The authors declare no competing financial interest and no conflicts of interest concerning the authorship and/or publication of this article.

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