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Neutrophil/Lymphocyte Ratio after Flow Citometry Periferic Blood Cell Detection - Predictive Marker of Anastomotic Fistula in Colorectal Cancer Surgery

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Abstract: *Anastomotic fistula is a dreadful complication of colon and rectal surgery that can put life into danger, being common after colorectal surgery. The preoperative lymphocyte neutrophil ratio (NLR) is known as a prognostic marker for colorectal cancer patients. The existence of a predictive marker of anastomotic fistula in colorectal cancer patients is not fully undestood, so we proposed to investigate the utility of preoperative NLR as a predictor of anastomotic fistula formation. This study the Neutrophils and lymphocytes were detected from periferic blood using flow citometry. We retrospectively evaluated 161 patients with colorectal cancer, who were treated curatively, in which at least one anastomosis was performed, comparing NLR values between patients who had fistula and those with normal healing, then comparing the group with low NLR, with the group with increased NLR, after finding the optimal value of NLR using the ROC curve.The optimal value of the NLR after establishing the cutoff value was 3.07. Between the low NLR group (n=134) and the high NLR group (n=27), were observed statistically significant differences in fistula (p <0.001) and death (p=0.001). The odds ratio for failure in the group with increased NLR was 10.37, which means that patients with NLR>3.54 have a chance of developing anastomotic fistula greater than 10.37 comparable to patients with lower NLR. We suggest the preoperative use of NLR can be used as a predictive marker of anastomotic fistula than can increase the quality of preoperative preparation and therefore the establishment of the optimal surgical technique that can lead to anastomotic fistula risk decrease.*

Keywords*:anastomotic, fistula, gastric cancer, predictive, preoperative, NLR*

1.Introduction

Anastomotic fistula is a major complication after curative surgery for colorectal cancer, being life threatening, increasing the risk of local recurrence and affecting the cancer long-term survival [1-3] .

The anastomotic dehiscence rate for the colon and rectum procedures varies between 5 and 21%, having a significant impact on the patient's health, with the mortality rate up to 27% [4,5] . The mortality rate is due to the increased septicity of the colonic contents that reach the peritoneum or pelvis that can lead to SIRS and sepsis.

Often, these leaks do not immediately are externalized on the drainage tube, or even if they are externalized, they also create a local collection, which maintains sepsis.

Given the ERAS (Enhanced Recovery After Surgery) recommendations and the increasingly supportive literature supporting drainage withdrawal after colo-rectal surgery, the need for predictive markers of fistula risk becomes even greater.

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The link between the development and evolution of the malignant process and systemic inflammation has been studied since the last century, with an estimated 25% of cancers are associated to systemic inflammation [6].

In recent years there have been numerous studies, which aimed to evaluate systemic inflammation and the body's response to it, in order to classify cancer patients. Currently, there is high evidence that C-reactive protein and albumin are markers of systemic inflammation in cancer patients [7]. The systemic inflammatory response is associated with changes in the circulation of white blood cells, with the presence of moderate neutrophilia and lymphopenia [8-10], thus establishing hematological tests that can be an easily measurable objective parameter, capable of expressing the severity of the systemic inflammatory response in patients with cancer.

From the point of view of biological phenomena involved in the healing of digestive anastomoses, neutrophils are recruited from the ischemic area and can cause the release of inflammatory mediators such as proteolysis enzymes and oxygen free radicals in the affected area [11,12], and lymphocytes play a role important in healing and reducing the effects of inflammation [12,13].

Given the above, we start from the hypothesis that NLR may also play a role in healing or delaying anastomosis healing, so we propose to study NLR as a correlation with the risk of anastomotic dehiscence.

Management of anastomotic fistulas usually involves more surgical procedures, colostomy creation and prolongation of hospitalization, so the existence of recognized predictive factors can improve management through early diagnosis and intervention.

Predicting the healing outcome in colic surgery can lead to better intervention and lower rates of anastomotic fistula. The neutrophil-lymphocyte ratio has recently been shown to be a predictor of overall mortality and response to chemotherapy in cancer patients [14-16].

The neutrophil / lymphocyte ratio is a simple marker, which can be easily calculated, based on the number of differentiated leukocytes, being recently established to be an indicator of systemic inflammatory status in the general population [17]. This ratio is considered to be a better indicator than leukocytes or the number of neutrophils for systemic inflammation [18].

Based on these data from the literature, we conducted the current study to investigate NLR as a measure of systemic inflammation and its relationship, if any, with anastomotic fistula. The inflammatory response plays an important role in the healing process, so its prolongation due to pre-existing inflammation can increase the susceptibility to anastomotic fistula.

2.Materials and methods

The study was approved by the ethics committee of the County Clinical Hospital of Craiova Emergency and this included patients diagnosed with colon and rectal cancer admitted to the Surgery Clinic I. Observation sheets of the 161 patients with colon and rectal cancer underwent surgery and involved at least one anastomosis in the intestine were analyzed prospective from January 2016 to December 2019, taking into account demographic data (sex, age), staging, histological type, leukocyte number, platelets, serum proteins , neutrophil / lymphocyte ratio and surgery.

In this study, the neutrophils and lymphocytes were detected from periferic blood using flow citometry.

Flow cytometry is a modern, complex and efficient technique of cell analysis, which allows the simultaneous determination of several physical and biochemical parameters characteristic of a single cell moving in a liquid current.

Inclusion criteria

The patients included in the study are the patients whose diagnosis was confirmed histo-pathologically from January 2016 to December 2019, with preoperative counting of blood figurative elements, before any hydroelectrolytic or protein balancing treatment is performed and those who have have completed the chemotherapy, radiotherapy or combined treatment.

Patients who were excluded from the study were those who were operated on urgently, those who had metastases with other locations, those with other concomitant malignancy, those with chronic anti-inflammatory treatment, those with incomplete oncological treatment and those with obvious evidence of systemic inflammation and autoimmune disease.

Statistical analysis

Data analysis was performed using SPSS version 20 with the establishment of descriptive statistics of the studied population.

The optimum value was established following the analysis of the characteristic ROC curve (figure 1), establishing a normal maximum limit in order to maximize the specificity and sensitivity in predicting anastomosis healing.

The independent t test was performed to determine whether the appearance of the fistula is related to the neutrophil-lymphocyte ratio.

We considered the results to be statistically significant if the P values on the two groups were <0.05, with a 95% confidence interval.

2.3. Study design

We performed blood cell counts on admission and calculated by dividing the percentage of neutrophils to the percentage of lymphocytes.

Numerical data were summarized as mean standard deviation and categorical variables were summarized as frequency (percentage)

The results were expressed as the value of the standard deviation ± the arithmetic mean for continuous quantitative variables. Quantitative variables were expressed as proportions. Univariate statistical analysis was performed using hypothesis confirmation tests: the chi-square test for qualitative variables, the Student t test for comparing quantitative variables with the homogeneity of variations in the Levene test.

3. Results and discussions

The study included a number of 161 patients (n = 161) who were chosen using the inclusion and exclusion criteria.

The average age of the patients was 68.19 ± 9.8 years, ranging from 37 to 92 years, with a percentage of male of 64.59% and a family history of cancer of 8.7%.

After the diagnosis the tumors were diagnosed as follows: Stage I (10.55%), stage II (34.16%), stage III (43.47%), stage IV 11.8%.

Leukocytes had values between 4310 and 20850 / mmc with an average of 8260 ± 2950. Hemoglobin was between 4.4 and 17.4 g / dL with an average of 11.85 ± 2.43.

Neutrophils had values between 2.15 and 16.81 with a mean of 5.30 ± 2.35.

Lymphocytes ranged from 0.34 to 7.20 with a mean of 2.11 ± 0.79.

NLR varied in all patients between 0.74 and 11.47. The mean value was 2.74 ± 1.44. The total proteins had values between 4.10 and 8.00 with a mean of 6.41 ± 0.68.

Platelets had values between 58,000 and 660,000 with an average of 282,476 ± 114,578

The link between the NLR and the failure of complete anastomosis healing was established, so we divided the patients studied into two groups: (1) the patients who performed the anastomotic dehiscence and the patients who had a complete anastomosis healing, without complications (2).

Table 1. Variations between high NLR group and low NLR group

	Total	NLR high (n=34)	NLR low (n=127)	*p* value
Age	68.19±9.80	64.85±11.75	69.08±9.05	0.058
Genre%				0.414
Male	104	24	80	
Females	57	10	47	
Dehiscence				<0.001*
Yes	27	23	4	
No	134	11	123	
Stage				0.023*
I	17	1	16	
II	55	12	43	
III	70	12	58	
IV	19	9	10	
Platelets	282.47±114.57	280.29±120.02	283.06±113.56	0.901
Leukocytes	8.26±2.95	9.77±4.15	7.86±2.40	0.014*
Hemoglobin	11.85±2.43	11.35±2.37	11.99±2.44	0.179
Total proteins	6.41±0.68	6.33±0.75	6.43±0.67	0.460
Death	6	5	1	0,002*

** p <0.05-statistically significant - independent t test with homogeneity of variations in Levene test*

To highlight the link between anastomotic dehiscence and NLR, the t-test was found to be statistically significant (p = 0.001), showing that there is a statistical difference in the NLR level between the patients with anastomotic dehiscence and the normal healing group of the anastomosis.

We found that the NLR is increased proportionally with the stage, so we performed the One-way ANOVA test to test the link between the NLR and the stage. The test showed that NLR and TNM status are dependent factors.

Setting the cutoff values of the NLR of high or low

The NL ratio is an inflammatory marker that best predicted the occurrence of anastomotic fistula with a surface over the Receiver Operating Characteristic ROC curve of 0.969 [0.945-0.992] (p <0.001) (Figure 1).

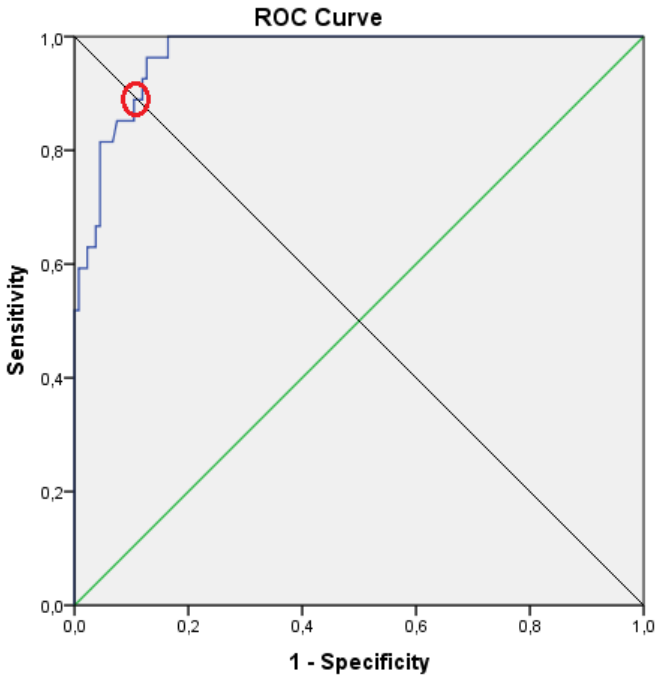

Figure 1. The characteristic ROC curve

The cutoff value of the ratio was set at 3.07, with a specificity of 91.2% and a sensitivity of 85.2%. Patients were classified into two groups according to the preoperative lymphocyte neutrophil ratio (<3.07 vs.> = 3.07), comprising on the one hand the patients who had fistula and on the other the patients who had a complete anastomosis healing (Figure 2). We summarized the variables in the low (n = 127) and high (n = 34) group of the NLR, compared with the whole study group (Table 2).

To determine the connection between anastomotic dehiscence and NLR level, we performed the Pearson Chi Square test in which we tested the occurrence of dehiscence in groups with low NLR and increased NLR, with a statistically significant result (p <0.001) with odd ratio (chance) of 10, 37, thus patients with NLR equal to or greater than 3.07, have 10.37 times more statistically chances of doing anastomotic dehiscence.

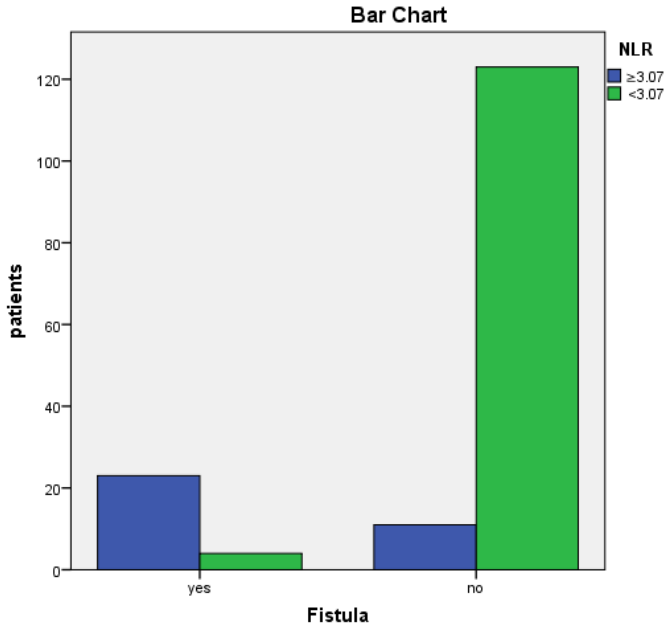

Figure 2. Correlation between fistula and NLR

In the group of patients with mean NLR anastomotic dehiscence it was 5.10 ± 2.02, and in the group of patients with complete anastomosis the average NLR anastomosis was 2.27 ± 0.60 Table 1 (variables between patients who did dehiscence and those with normal healing).

Table 2. Variations between the group that made the fistula and the group with normal healing

	Anastomotic fistula (n=27)	Normal healing (n=134)	p value
Age	67.37±12.55	68.35±9.20	0.702
Genre %			0.495
Male	19	85	
Female	8	49	
NLR	5.10±2.02	2.27±0.60	<0.001*
Stage			
I	1	16	0.006*
II	7	48	
III	11	59	
IV	8	11	
Platelets	255.14±105.22	287.98±115.95	0.175
Leukocytes	9.59±3.98	7.99±2.63	0.055
Hemoglobin	11.79±2.50	12.17±2.07	0.465
Total proteins	6.20±0.79	6.46±0.65	0.077
Death	5	1	0.004*

** p <0.05-statistically significant - independent t test with homogeneity of variations in Levene test*

Significance of the neutrophil lymphocyte ratio

The link between cancer and inflammation was initially proposed by Virchow in 1863 when he found the presence of leukocytes in neoplastic cells [19,20]. There is ample evidence that a progression of cancerous disease is the result of the interaction between the local character of the tumor and the systemic inflammation of the host [21,22]. Systemic inflammation may also play an important role in carcinogenesis as well as in local and general cancer invasion [23].

In the last decade, it has been hypothesized that hematological markers of the systemic inflammatory response predict survival, the most widely used being NLR according to unost studies performed for a variety of colorectal tumors at different stages. Hypothetically, NLR may represent an indicator of the balance of the host's systemic inflammation [9].

Observations from numerous studies have shown that naturally the number of neutrophils and lymphocytes is inversely proportional, translating the balance between inflammation and angiogenesis (large number of neutrophils) and the host's favorable immune response (large number of lymphocytes), demonstrating that, large numbers of neutrophils are associated with lower overall survival while higher levels of lymphocytes are correlated with better prognosis and higher survival.

Systemic inflammatory response can be measured by using the NLR surrogate as a cheap and widely available marker to predict the survival of cancer patients [24]. Several studies have shown that increased NLR is associated with decreased survival in several cancers, most of which refer to colorectal cancer [25 - 27]. The causes of this low survival have not yet been identified, instead an increased NLR is considered to be correlated with decreased nutrition and immune status, more precisely some studies have shown that for advanced rectal cancer the increased number of lymphocytes is associated with an increase in the number of patients with partial remission following

neuroadjuvant chemioradiotherapeutic treatment [28], while increased lymphocyte neutrophil ratio is associated with shorter local recurrence time and poorer overall survival [29].

The relationship between increased NLR, poor prognosis of patients and advanced stage of the tumor may be explained by the impaired immune response of the host that is dependent on leukocytes [9].

Cell-mediated immunity may be reflected by lymphocyte count, while neutrophils reflect systemic inflammation [30], so NLR calculation by dividing the number of neutrophils by lymphocyte count may be an indicator of the type of host response to the tumor.

NLR is an acute phase reactant and can be considered a new indicator in the formation of anastomotic fistula, even predicting early mortality. This is the first study to predict preoperatively the formation of anastomotic fistula in patients with increased NLR who have undergone surgery for colon cancer.

Anastomosis causes tissue damage where the suture is performed, stimulating in this situation a rapidly coordinated physiological response to stimulate hemostasis, followed by inflammation, proliferation and remodeling. After anastomosis, neutrophils are the first to initiate the healing process with the onset of inflammatory phase, which have increased motility, infiltrating the wound within one hour of injury, reaching sustained levels at 48 hours [31]. Neutrophils have the role of destroying foreign particles and bacteria at the level of the anastomotic line by phagocytosis followed by degranulation with the release of toxic substances (proteases, cathepsin, lactoferin) which have the role of destroying bacteria and dead tissue in that area. It has recently been shown that neutrophils can also produce chromatin and protease traps that capture and kill bacteria from extracellular spaces. Necrotic residues, foreign matter, and captured bacteria generate oxygen free radicals that can be combined with chlorine to sterilize the wound [32]. Neutrophils are probably the least studied inflammatory cells in colorectal cancer, so the role of neutrophil infiltration in its prognosis has been the subject of conflicting reports, indicating a protective role and an aggravating role [33-35].

Excessive infiltration and / or retention of neutrophils in the wound has a subsequent effect on healing by release of oxidants and hydrolytic enzymes from activated neutrophils [36]. It has been reported that the persistence of activated neutrophils in the wound would suppress macrophage activity, having a direct effect on bacterial clearance [37].

Lymphocytes appear after 72 hours and are considered to be important in regulating the wound healing process by producing an extracellular matrix and reshaping collagen. Experimental studies have shown that inhibition of T lymphocytes results in decreased wound resistance and impaired collagen deposition [38].

The inflammatory phase of healing will normally persist as long as it is needed, in order to destroy all bacteria and to remove excess tissue residues from the anastomotic line. Prolonged inflammation induced by pre-existing inflammatory status can lead to further tissue injury, delayed proliferation, leading to the formation of a chronic wound [39], resulting in anastomotic dehiscence in our case.

From our data, we aimed to demonstrate that NLR was significantly increased in patients who had anastomotic dehiscence after colorectal cancer surgery, which involved at least one anastomosis. The mean NLR in the group of patients who had anastomotic dehiscence was increased to 5.10 ± 2.02 compared to the group that had a complete complication of anastomosis without complication, which averaged 2.27 ± 0.60. The difference between the average of the two groups was statistically significant in the independent t test ($p < 0.001$), proving that patients with anastomotic dehiscence had an increased level of NLR.

Some NLR studies independent of TNM.

Currently, numerous studies have shown that inflammation associated with tumors and tumor microenvironment can play an important role in the development, progression and metastasis of cancer [40-42]. The inflammatory response in the tumor environment is reflected by common markers found in the peripheral blood such as cytokines, leukocytes and their subtypes, thus increased lymphocyte

levels being a good indicator of cancer prognosis [43-45]. Numerous observational studies have shown that increased NLR correlates with advanced tumor stage and also low prognosis in a variety of cancers in the breast [46,47], kidney [48,49], nasopharyngeal [50,51], head and neck [52,53]. NLR values between 3 and 5 in colorectal cancer patients have been associated with poorer postoperative outcomes, burdened by numerous complications, so preoperative NLR evaluation may allow optimization of therapeutic strategy in patients with operable colon cancer [54-57], being used so far only as a risk marker for postoperative complications and prognosis of cancerous disease.

Analyzing the collected data it was observed that the NLR is higher as the colorectal tumor stage increases, so we applied the One-way ANOVA test to determine exactly whether the NLR is stage dependent. The p-value determined in the One-way ANOVA test was = 0.001 which shows that from a statistical point of view the NLR value is independent of the tumor stage.

We set the cutoff value to define what is the low value of the NLR and the increased value of the NLR in order to allow the most correct classification of patients with fistula risk. From the ROC curve it was found that the NLR value of 3.07 is the most appropriate, having statistical significance ($p < 0.001$). This value of NLR is very close to other studies that have discussed postoperative complications and survival in colorectal cancer [58-60].

When dividing the batch into two groups, one with high NLR and the other with low NLR observed that sex ($p = 0.414$), age ($p = 0.058$), platelet count ($p = 0.901$) and biochemical determinations of hemoglobin ($p = 0.179$) and total proteins ($p = 0.460$) did not show a statistically significant difference between the two groups. There were differences between the two groups in terms of stage ($p = 0.023$), death ($p = 0.002$), leukocyte number ($p = 014$) and anastomotic dehiscence ($p < 0.001$) indicating that most patients with NLR they were older and had a high risk of anastomotic dehiscence and death.

The odds ratio (OR) for failure in the group with increased NLR was 10.37, which means that patients with NLR greater than 3.07 have a 10.37 greater chance of performing anastomotic dehiscence than patients with NLR less than this value.

Comparing the group of patients with anastomotic dehiscence with the group who had normal anastomosis healing, the statistically significant difference was found at NLR ($p < 0.001$), stage ($p = 0.006$), and patients who died ($p = 0.004$), over time what sex ($p = 0.495$), age ($p = 0.702$), platelet count ($p = 0.175$), total protein ($p = 0.077$,) leukocytes ($p = 0.055$) and hemoglobin ($p = 0.465$) did not register, statistically significant difference.

In view of these observations it is possible to propose and use this hematological test as a predictive marker of anastomotic dehiscence in colorectal cancer patients, helping to better stratify the patients and helping to establish the optimum time for surgical treatment.

4.Conclusions

Analyzing the data of the study we conclude that an increased preoperative NLR (> = 3.07) is a good negative indicator of anastomotic dehiscence with a specificity of 91.2% and a sensitivity of 85.2%, being correlated with the appearance of anastomotic fistula in patients with anastomosis in the large intestine after colon or rectal cancer.

Patients with NLR equal to or greater than 3.07, have 10.37 times more statistically chances of doing anastomotic dehiscence.

The inflammatory status reflected by increased NLR can delay the healing process, forming a chronic wound, leading to anastomotic dehiscence.

This study also compared the association between mortality and NLR, which was significantly higher in patients who died after surgery.

We consider that preoperative NLR can be used as a predictive marker of anastomotic fistula than can increase the quality of preoperative preparation and therefore the establishment of the optimal surgical technique that can lead to anastomotic fistula risk decrease.

References

1.PETERSEN, S., FREITAG, M., HELLMICH, G., LUDWIG, K., Anastomotic leakage: impact on local recurrence and survival in surgery of colorectal cancer, *Int J Colorectal Dis*, **13**, 1998,160-163.
2.BELL, S.W., WALKER, K.G., RICKARD, M.J., et al. Anastomotic leakage after curative anterior resection results in a higher prevalence of local recurrence, *Br J Surg*, **90**, 2003,1261-1266.
3.BRANAGAN, G., FINNIS, D., Prognosis after anastomotic leakage in colorectal surgery. *Dis Colon Rectum*, **48**, 2005, 1021-1026.
4.FOUDA, E., EL NAKEEB, A., MAGDY, A., et al., Early detection of anastomotic leakage after elective low anterior resection, *J Gastrointest Surg*, **15**, 2011, 137–144.
5.TRENCHEVA, K., MORRISSEY, K.P., WELLS, M., et al., Identifying important predictors for anastomotic leak after colon and rectal resection: prospective study on 616 patients, *Ann Surg* **257**, 2013, 108–113
6.BALKWILL, F.R., MANTOVANI, A., Cancer-related inflammation: common themes and therapeutic opportunities, *Semin Cancer Biol*. **22**, 2012, 33-40.
7.ROXBURGH, C.S., MCMILLAN, D.C., Role of systemic inflammatory response in predicting survival in patients with primary operable cancer, *Future Oncology*, **6(1)**, 2010, 149–63.
8.GABAY, C, KUSHNER, I., Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine* **340(6)**, 1999 , 448–454.
9.ZAHOREC, R., Ratio of neutrophil to lymphocyte counts – rapid and simple parameter of systemic inflammation and stress in critically ill, *Bratislavske Lekarske Listy* **102(1)**, 2001, 5–14.
10.ZHEN, BIAN., YANAN, GUO., BINH, H.A., KE ZEN, YUAN, LIU, Regulation of the inflammatory response: enhancing neutrophil infiltration under chronic inflammatory conditions - *J Immunol*, **188(2)**, 2012 Jan 15, 844–853.
11.HARTL, R., SCHURER, L., SCHMID-SCHONBEIN, G.W. et al., Experimental antileukocyte interventions in cerebral ischemia, *J Cereb Blood Flow Metab* **16**, 1996, 1108-1119.
12.FRANGOGIANNIS, N.G., SMITH, C.W., ENTMAN, M.L., The inflammatory response in myocardial infarction, *Cardiovasc Res*, **53**, 2002, 31-47.
13.SCHWARTZ M., MOALEM, G., Beneficial immune activity after CNS injury: prospects for vaccination, *J Neuroimmunol*, **113**, 2001, 185-192.
14.DIRICAN, N., KARAKAYA, Y.A., GUNES, S., DALOGLU, F.T., DIRICAN, A., Association of intratumoral tumor infiltrating lymphocytes and neutrophil-to-lymphocyte ratio are an independent prognostic factor in non-small cell lung cancer, *Clin Respir J*, **11(6)**, 2017, 789-796
15.HASEGAWA, S., EGUCHI, H., TOMOKUNI, A., TOMIMARU, Y., ASAOKA, T., WADA, H., et al. Pre-treatment neutrophil to lymphocyte ratio as a predictive marker for pathological response to preoperative chemoradiotherapy in pancreatic cancer, *Oncol Lett*, **11**, 2016, 1560-1566.
16.NAGASAKI, T., AKIYOSHI, T., FUJIMOTO, Y., KONISHI, T., NAGAYAMA, S., FUKUNAGA, Y., et al., Prognostic impact of neutrophil-tolymphocyte ratio in patients with advanced low rectal cancer treated with preoperative chemoradiotherapy, *Dig Surg*, **32,** 2015, 496-503.
17.IMTIAZ, F., SHAFIQUE, K., MIRZA, SS., et al., Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. *Int Arch Med* **5** 2012, doi: 10.1186/1755-7682-5-2
18.BHAT, T., TELI, S., RIJAL, J., et al. Neutrophil to lymphocyte ratio and cardiovascular diseases: a review. *Expert Rev Cardiovasc Ther* **11**, 2013, 55-59.
19.BALKWILL, F., MANTOVANI, A., Inflammation and cancer: back to Virchow?, *Lancet*, **357**, 2001, 539–545.
20.GARCEA, G., LADWA, N., NEAL, C.P., METCALFE, M.S., DENNISON, A.R., BERRY, D.P., Preoperative neutrophil-to-lymphocyte ratio (NLR) is associated with reduced disease-free survival following curative resection of pancreatic adenocarcinoma, *World J Surg*, **35,** 2011, 868–872.
21.MANTOVANI, A., ROMERA, P., PALUCKA, A.K., et al., Tumor immunity: effector response to tumor and role of the microenvironment, *Lancet*, **371**, 2008, 771–783.

22.ROXBURGH, C.S.D., MCMILLAN, D.C., Role of systemic inflammatory response in predicting survival in patients with primary operable cancer, *Future Oncol*, **6**, 2010, 149–163.
23.WALSH, S.R., COOK, E.J., GOULDER, F., JUSTIN, T.A., KEELING, N.J., Neutrophil-lymphocyte ratio as a prognostic factor in colorectal cancer, *J Surg Oncol*, **91**, 2005, 181-184.
24.GUTHRIE, G.J., CHARLES, K.A., ROXBURGH, C.S., HORGAN, P.G., MCMILLAN, D.C., CLARKE, S.J., The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer, *Crit Rev Oncol Hematol*, **88**, 2013, 218–230.
25.MALIETZIS, G., GIACOMETTI, M., ASKARI, A., NACHIAPPAN, S., KENNEDY, R.H., FAIZ, O.D., AZIZ, O., JENKINS, J.T. A preoperative neutrophil to lymphocyte ratio of 3 predicts disease-free survival after curative elective colorectal cancer surgery, *Ann Surg*, **260,** 2014, 287–292.
26.ABSENGER, G., SZKANDERA, J., PICHLER, M., STOTZ, M., ARMINGER, F., WEISSMUELLER, M., SCHABERL-MOSER, R., SAMONIGG, H., STOJAKOVIC, T., GERGER, A., A derived neutrophil to lymphocyte ratio predicts clinical outcome in stage II and III colon cancer patients, *Br J Cancer*, **109**, 2013, 395–400.
27.ISHIZUKA, M., NAGATA, H., TAKAGI, K., IWASAKI, Y., KUBOTA, K., Combination of platelet count and neutrophil to lymphocyte ratio is a useful predictor of postoperative survival in patients with colorectal cancer, *Br J Cancer*, **109**, 2013, 401–407.
28.KITAYAMA, J., YASUDA, K., KAWAI, K., SUNAMI, E., NAGAWA, H., Circulating lymphocyte number has a positive association with tumor response in neoadjuvant chemoradiotherapy for advanced rectal cancer. *Radiat Oncol*, **5**, 2010, 47, doi:10.1186/1748-717X-5-47
29.CARRUTHERS, R., THO, L.M., BROWN, J., KAKUMANU, S., MCCARTNEY, E., MCDONALD, A.C., Systemic inflammatory response is a predictor of outcome in patients undergoing preoperative chemoradiation for locally advanced rectal cancer, *Colorectal Dis*, **14**, 2012, 701–707.
30.LI, M.X., LIU, X.M., ZHANG, X.F., ZHANG, J.F., WANG, W.L., ZHU, Y., et al., Prognostic role of neutrophil- to-lymphocyte ratio in colorectal cancer: A systematic review and meta-analysi, *Int J Cancer*, **134**, 2014, 2403-2413.
31.BROUGHTON, G., JANIS, J.E., ATTINGER, C.E., The basic science of wound healing. *Plast Reconstr Surg*, **117**(7 Suppl) 2006 Jun, 12S-34S.
32.VELNAR T., BAILEY T., SMRKOLJ V., The wound healing process: an overview of the cellular and molecular mechanisms, *J Int Med Res*, **37**, 2009 Sep-Oct, 1528-1542.
33.DROESER, R.A., HIRT, C., EPPENBERGER-CASTORI, S., et al., High myeloperoxidase positive cell infiltration in colorectal cancer is an independent favorable prognostic factor, *PLoS One*, **8(5)**, 2013, e64814
34.GALDIERO, M.R., BIANCHI, P., GRIZZI, F., et al., Occurrence and significance of tumor-associated neutrophils in patients with colorectal cancer, *Int J Cancer*, **139**, 2016, 446-456
35.RAO, H.L., CHEN, J.W., LI, M., et al., Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis *PLoS One*, **7**, 2012, e30806
36.LIU, Y., COUSIN, J.M., HUGHES, J., VAN, DAMME, J., SECKL, J.R., HASLETT, C., DRANSFIELD, I., SAVILL, J., ROSSI, A.G., Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes. *J. Immunol*. 1999, **162**, 3639–3646.
37.SHAILENDRA, SINGH, ALISTAIR, YOUNG, CLARE-ELLEN MCNAUGHT, The physiology of wound healing, *Surgery - Oxford International Edition*, **35(9)**, August 2017, 473 – 477
38.PETERSON, J.M., BARBUL, A., BRESLIN, R.J., WASSERKRUG, H.L., EFRON, G., Significance of T-lymphocytes in wound healing, *Surgery*, **102**, 1987, 300-305.
39.NATHAN, C. Points of control in inflammation. *Nature* **420** 2002 Dec 846-852.
40.GALDIERO, M.R., BONAVITA, E., BARAJON, I., GARLANDA, C., MANTOVANI, A., JAILLON, S., Tumor associated macrophages and neutrophils in cancer, *Immunobiology*, **218**, 2013, 1402–1410.

41.NOY, R., POLLARD, J.W., Tumor-associated macrophages: from mechanisms to therapy, *Immunity,* **41**, 2014, 49–61.
42.VAN, VERSCHUER, V.M., HOONING, M.J., VAN, BAARE-GEORGIEVA, R.D., HOLLESTELLE, A., TIMMERMANS, A.M., KOPPERT, L.B., et al., Tumor-associated inflammation as a potential prognostic tool in BRCA1/2-associated breast cancer, *Hum Pathol,* **46**, 2015, 182–190.
43.MEI, Z., LIU, Y., LIU, C., CUI, A., LIANG, Z., WANG, G., et al., Tumour-infiltrating inflammation and prognosis in colorectal cancer: systematic review and meta-analysis, *Br J Cancer,* **110**, 2014, 1595–605.
44.YEUNG, A.W.K., TZVETKOV, N., EL-TAWIL, O.S., BUNGAU, S.G., ABDEL-DAIM, M.M., ATANASOV, A.G., Antioxidants: scientific literature landscape analysis, *Oxid. Med. Cell. Longev.*, **2019**, 2019, 11 pages. https://doi.org/10.1155/2019/8278454
45.GAMAN, A.M., MOISA, C., DIACONU, C.C., GAMAN, M.A., Crosstalk between oxidative stress, chronic inflammation and disease progression in essential thrombocytemia. *Rev. Chim.***70**(10), 2019, 3486-3489.
46.KOH, C.H., BHOO-PATHY, N., NG K.L., JABIR, R.S., TAN, G.H., SEE, M.H., et al., Utility of pre-treatment neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as prognostic factors in breast cancer, *Br J Cancer*, **113**, 2015, 150-158.
47.KRENN-PILKO, S., LANGSENLEHNER, U., STOJAKOVIC, T., PICHLER, M., GERGER, A., KAPP, K.S., et al., The elevated preoperative derived neutrophilto- lymphocyte ratio predicts poor clinical outcome in breast cancer patients, *Tumor Biol*, **37**, 2016, 361-368.
48.HU, K., LOU, L., YE, J., ZHANG, S., Prognostic role of the neutrophil-lymphocyte ratio in renal cell carcinoma: a metaanalysis, *BMJ Open*, **5** 2015, e006404.
49.DE, MARTINO, M., PANTUCK, A.J., HOFBAUER, S., WALDERT, M., SHARIAT, S.F., BELLDEGRUN, A.S., et al., Prognostic impact of preoperative neutrophil-to-lymphocyte ratio in localized nonclear cell renal cell carcinoma, *J Urol*. **190**, 2013, 1999-2004.
50.HE, J.R., SHEN, G.P., REN, Z.F., QIN, H., CUI, C., ZHANG, Y., et al., Pretreatment levels of peripheral neutrophils and lymphocytes as independent prognostic factors in patients with nasopharyngeal carcinoma, *Head Neck*, **34**, 2012, 1769-1776.
51.JIN, Y., YE, X., HE, C., ZHANG, B., ZHANG, Y., Pretreatment neutrophil-to-lymphocyte ratio as predictor of survival for patients with metastatic nasopharyngeal carcinoma. *Head Neck*, **37**, 2015, 69-75.
52.HADDAD, C.R., GUO, L., CLARKE, S., GUMINSKI, A., BACK, M., EADE, T., Neutrophil-to-lymphocyte ratio in head and neck cancer, *J Med Imaging Radiat Oncol*, **59**, 2015, 514-519.
53.RACHIDI, S., WALLACE, K., WRANGLE, J.M., DAY, T.A., ALBERG, A.J., LI, Z., Neutrophil-to-lymphocyte ratio and overall survival in all sites of head and neck squamous cell carcinoma, *Head Neck*, **38**, 2016, E1068-1074.
54.HUNG, H.Y., CHEN, J.S., YEH, C.Y., CHANGCHIEN, C.R., TANG, R., HSIEH, P.S., et al., Effect of preoperative neutrophil–lymphocyte ratio on the surgical outcomes of stage II colon cancer patients who do not receive adjuvant chemotherapy., *Int J Colorectal Dis*, **26**, 2011, 1059-1065.
55.MALIETZIS, G., GIACOMETTI, M., ASKARI, A., NACHIAPPAN, S., KENNEDY, R.H., FAIZ, O.D., et al., A preoperative neutrophil to lymphocyte ratio of 3 predicts disease-free survival after curative elective colorectal cancer surgery, *Ann Surg*, **260**, 2014, 287-292.
56.NEDELCUTA, R.M., CALIN, G., BALEANU, V.D., DAVITOIU, D.V., VOICULESCU, D.I., MIREA, C.S., TENEA COJAN, T.S., SOCEA, B., ALEXANDRU, D.O., CLENCIU, D., GHEORMAN, V., UDRISTOIU, I., CALBOREAN, V., CIORA, C.A.: Anorexia, a First Sign of Diagnosis in Severe Pediatric Disorders, *Rev.Chim.*, **70**(5), 2019, 1698-1702
57.DRAGHICI, T., NEGREANU, L., BRATU, O.G., PANTEA STOIAN, A., SOCEA, B., NEAGU, T.P., STANESCU, A.M.A., MANUC, D., DIACONU, C.C. Paraneoplastic syndromes in digestive tumors: a review, *Rom Biotechnol Lett*. **24**(5), 2019, 813-819.

58.ZAHA, D.C., BUNGAU, S., ALEYA, S., TIT, D.M., et al. What antibiotics for what pathogens? The sensitivity spectrum of isolated strains in an intensive care unit, *Sci Total Environ*. **687**, 2019, 118-127.
59.NEDELCUTA, R., POPESCU, M., CALIN, G.: Digestive Manifestations in Wilms' Tumor. NEUROGASTRO 2017 - Meeting Of The Romanian Society Of Neurogastroenterology With Rome Iv Regional Central East European Meeting, 2017, 200-204.
60.PINE, J. K. et al., Systemic neutrophil-to-lymphocyte ratio in colorectal cancer: the relationship to patient survival, tumour biology and local lymphocytic response to tumour, *Br J Cancer*, **113**, 2015, 204–211, doi: 10.1038/bjc.2015.87

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