THE ROLE OF COMPLETE BLOOD COUNT PARAMETERS IN PATIENTS WITH COLORECTAL CANCER

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SUMMARY – Chronic inflammation has been linked with many cancers. It seems that easily available and usual blood inflammatory markers might serve as a prognostic factor for overall survival and disease-free survival in patients with various cancers. Preoperative neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), as well as hemoglobinemia, thrombocytosis, elevated C-reactive protein values, neutropenia and leukocytosis have been shown to affect overall survival and disease-free survival in patients with colorectal cancer (CRC), however, with controversial results. Complete blood count, NLR and PLR were determined in 71 patients with CRC (stages 3 and 4) after neoadjuvant chemo-radiotherapy and before surgery, treated at Hospital for Tumors in Zagreb. Statistical analysis included Mann-Whitney U test, Student’s t-test, univariate and multivariate analysis. The results of Mann-Whitney U test and Student’s t-test showed that neutrophil count (p=0.024), NLR (p=0.003) and PLR (p=0.007) correlated significantly with overall survival. However, there was no significant correlation of age, leukocyte, lymphocyte and platelet counts and hemoglobin values with overall survival of patients. Furthermore, the same tests showed that leukocyte (p=0.04), neutrophil (p=0.0014) and platelet (p=0.006) counts, NLR (p=0.0006) and PLR (p=0.0015), as well as hemoglobin values (p=0.028) correlated significantly with disease-free survival. The results of univariate analysis showed that unlike PLR, NLR correlated with overall survival and disease-free survival (p=0.0002), although the correlation of PLR and disease-free survival almost reached significance (p=0.059). Furthermore, the results of univariate analysis showed significant correlation of advanced pathological TNM stage with overall survival. There was no correlation of patient age and gender, tumor stage and neoadjuvant chemo-radiotherapy with overall survival and disease-free survival. The results of multivariate analysis showed that NLR (cut-off value 3.27) and advanced pathological TNM stage significantly correlated with disease-free survival but not with overall survival. It seems that NLR might be an accurate marker for overall survival and disease-free survival in CRC patients after neoadjuvant chemo-radiotherapy and before surgery.

Key words: Inflammation; Blood cell count; Colorectal neoplasms; Disease-free survival; C-reactive protein

Introduction

Colorectal cancer (CRC) is the third cancer in Europe with fatal outcome. Currently, therapy for locally advanced CRC relies on neoadjuvant chemo-radiotherapy (CRT) in some patients in order to increase
the tumor-free resection margin. Inflammation within cancers might influence future behavior of malignant cells and blood vessels, as well as therapeutic response. Some serum biomarkers might give information on the level of systemic inflammation and determine the future course of disease in many cancer patients. Anemia, hemoglobinemia, thrombocytosis and elevated C-reactive protein (CRP) values are thought to be adverse prognostic factors in various malignancies. Recently, it has been reported that the neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) might indicate patients that will respond poorly to treatments (neoadjuvant CRT) and who will have worse disease-free survival (DFS) and overall survival (OS) in many cancers such as small cell lung carcinoma, esophageal carcinoma, pancreatic adenocarcinoma, and head and neck cancers. Systemic meta-analysis in CRC patients has shown that NLR has a prognostic value in terms of rational patient stratification for further treatment adjustment. As suggested by Kitayama et al., tumor shrinkage is dependent on tumor cells and host characteristics, therefore, there is a possibility that a lymphocyte-mediated immune reaction might result in partial or total eradication of tumor cells. Therefore, lymphocyte count in the circulation may improve response to therapy in CRC patients. However, the role of NLR and PLR in CRC after CRT and before surgery is yet to be established.

Materials and Methods

Prior to this study, participants signed informed consent according to Helsinki II. Initially, 150 charts of patients having undergone neoadjuvant CRT followed by surgery at the Hospital for Tumors, Sestre milosrdnice University Hospital Centre in Zagreb, were retrieved. Of these, 71 patient charts that included data on all study parameters were analyzed for complete blood count, lymphocyte, leukocyte, neutrophil and platelet counts, hemoglobin, NLR and PLR. NLR was calculated by dividing absolute neutrophil count by absolute lymphocyte count. PLR was calculated by dividing absolute platelet count by absolute lymphocyte count. Primary tumors were staged by use of magnetic resonance imaging (MRI). All patients were irradiated and administered concurrent 5-fluorouracil-based chemotherapy. The mean radiation dose was 45 Gy (range 45-50.4 Gy) with daily fraction of 1.8. Gy. Radiation treatment was performed according to the institutional protocols. Pathological tumor staging of surgical specimen was performed in accordance with the 7th Guidelines of the American Joint Committee on Cancer. The primary endpoint of the study was OS and DFS. OS was defined as the time from the date of surgery to death. DFS was defined as the time from the date of surgery to the date of tumor relapse (local recurrence and/or distant metastases) or death. The secondary endpoint was neoadjuvant chemoradiation response as measured by ypTNM staging. In the ypTNM staging system, good response was defined as ypT0 to 2 without lymph node metastases, and poor response was defined as ypT3 to 4 or with lymph node metastases. Statistical analysis included Mann-Whitney U test, Student’s t-test, univariate and multivariate analysis. The level of significance was set at p<0.05.

Results

The results of Mann-Whitney U test and Student’s t-test showed that neutrophil count (p=0.024), NLR (p=0.003) and PLR (p=0.007) correlated significantly with OS (Table 1). However, there was no significant correlation of age, leukocyte, lymphocyte and platelet counts, and hemoglobin values with OS. Furthermore,

| Characteristic | Overall survival | p value |
|---------------|-----------------|---------|
|               | Yes | No |         |
| Number of patients | 63  | 8  | -       |
| Age (yrs) | 61 (36-79) | 56.5 (34-79) | 0.68    |
| Leukocytes | 8.16±1.94 | 9.04±3.42 | 0.27    |
| Neutrophils | 5.13±1.6 | 6.64±2.77 | 0.024*  |
| Lymphocytes | 2.03±0.55 | 1.7±0.7 | 0.138   |
| Platelets | 310.7±97.58 | 397.12±239.9 | 0.059   |
| NLR | 2.76±1.27 | 4.36±2.18 | 0.003*  |
| PLR | 166.2±73.56 | 249.5±124.1 | 0.007*  |
| Hemoglobin | 129.1±22.2 | 118.7±26.87 | 0.23    |

*median with range; p value was calculated by Mann-Whitney-U test; mean ± standard deviation; differences between groups were analyzed by Student’s t-test; NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; *p value lower than 0.05 set as the level of significance.
Table 2. Age, complete blood count and disease-free survival in the study population

| Characteristic          | Disease-free survival | p value |
|------------------------|-----------------------|---------|
|                        | Overall survival      |         |
|                        | Yes                  | No      |
| Number of patients     | 50                   | 21      | -       |
| Age<sup>a</sup> (yrs)  | 61 (36-79)            | 59 (34-79) | 0.29    |
| Leukocytes<sup>b</sup> | 7.93±1.92             | 9.05±2.48 | 0.04*   |
| Neutrophils<sup>b</sup> | 4.86±1.44             | 4.79±2.19 | 0.0014* |
| Lymphocytes<sup>b</sup> | 2.07±0.58             | 1.8±0.54  | 0.08    |
| Platelets<sup>b</sup>  | 294.9±85.7            | 380.9±169.9 | 0.006*  |
| NLR<sup>b</sup>        | 2.56±1.17             | 3.84±1.75 | 0.0006* |
| PLR<sup>b</sup>        | 155.6±70.04           | 223.1±96.18 | 0.0015* |
| Hemoglobin<sup>b</sup> | 131.7±21.6            | 118.8±23.49 | 0.028*  |

<sup>a</sup>median with range; <sup>b</sup>mean ± standard deviation; differences between groups were analyzed by Student’s t-test; NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; *p value lower than 0.05 set as the level of significance

The same tests showed that leukocyte (p=0.04), neutrophil (p=0.0014) and platelet (p=0.006) counts, NLR (p=0.0006) and PLR (p=0.0015), as well as hemoglobin values (p=0.028) correlated significantly with DFS (Table 2). The results of univariate analysis showed that unlike PLR, NLR correlated with OS and DFS (p=0.0002); however, correlation of PLR and DFS almost reached significance (p=0.059). There was no correlation between patient age and gender, tumor stage and neoadjuvant CRT with OS and DFS (Table 3). The results of multivariate analysis showed that NLR (cut-off value 3.27) and advanced pathological TNM stage significantly correlated with DFS but not with OS (Table 4).

Discussion

Some studies demonstrated that preoperative NLR and PLR were significantly increased in CRC patients.

Table 3. Univariate analysis of age, adjuvant chemo-radiotherapy, tumor stage, NLR, PLR and platelets in the study population

| Patient characteristic          | Overall survival | Disease-free survival |
|---------------------------------|-----------------|-----------------------|
|                                 | HR 95% CI       | p-value               |
|                                 | HR 95% CI       | p-value               |
| Age<sup>a</sup> (yrs):          |                 |                       |
| <60                             | 1.09 0.27-4.4   | 0.89                  |
| >60                             |                 |                       |
| Sex:                            |                 |                       |
| female                          | 2.03 0.46-8.29  | 0.36                  |
| male                            |                 |                       |
| Adjuvant chemotherapy:          |                 |                       |
| no                              | 0.64 0.15-2.64  | 0.54                  |
| yes                             |                 |                       |
| Stage                           |                 |                       |
| 3                               | 2.89 0.22-169   | 0.29                  |
| 4                               |                 |                       |
| NLR                             |                 |                       |
| <3.27                           | 4.75 1.4-33.83  | 0.017*                |
| >3.27                           |                 |                       |
| Platelets                       |                 |                       |
| <300                            | 1.12 0.28-4.54  | 0.87                  |
| >300                            |                 |                       |
| PLR                             |                 |                       |
| <150                            | 2.05 0.47-8     | 0.36                  |
| >150                            |                 |                       |

NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; HR = hazard ratio; 95% CI = 95% confidence interval; *p value lower than 0.05 set as the level of significance
when compared to healthy controls. Furthermore, some authors suggest that NLR might be a useful, simple and inexpensive biomarker of response to CRT in patients with CRC. Malietzis et al. analyzed 13 studies which evaluated NLR as a sign of treatment outcome in patients with CRC. They report that a high pre-treatment NLR independently predicted shorter survival in all studies (HR 2.08; 95% CI 1.64-2.64). Furthermore, high NLR became a significant sign of poor outcome at years 2 and 3, but not in the first year of follow up. Furthermore, He et al. conclude that NLR and CRP were the only independent factors for OS and progression-free survival (PFS) in CRC patients. Additionally, Li et al. analyzed data from 16 studies in order to associate NLR with OS and PFS in CRC patients. Their analysis revealed that elevated pretreatment NLR correlated with poorer OS and PFS in CRC patients. Increased NLR significantly correlated with lower tumor differentiation and higher carcinoembryonic antigen level. Galizia et al. report that decreased NLR values correlated significantly with increased DFS; along with increased carcinoembryonic antigen levels and Dukes B stage, increased levels of NLR independently depicted worse prognosis in 503 CRC patients. This finding is in concordance with our results. Even in Dukes A patients, NLR differentiated between relapsing and non-relapsing patients. Preoperative NLR as an inexpensive and easily available biomarker might indicate tumor recurrence and should be implemented in tailored therapy in early stage of CRC. Onozawa et al. analyzed 83 patients administered oxaliplatin-based chemotherapy for stage IV CRC, divided into two groups according to NLR; the median survival time of patients with high NLR (≥3.0) was significantly worse than that of patients with low NLR (<3.0; 16.1 months vs. 25.4 months, p=0.03). Ying et al. studied preoperative NLR, PLR and lymphocyte-to-monocyte ratio in 205 surgical CRC patients. They concluded that preoperatively elevated NLR was an independent prognostic marker for recurrent-free survival, OS and cancer specific survival. However, Shen et al. report no significant correlation between NLR and response to neoadjuvant CRT. Nevertheless, the same authors conclude that an increased baseline NLR is a simple, inexpensive and available prognostic marker for OS together

Table 4. Multivariate analysis of adjuvant chemo-radiotherapy, tumor stage, NLR, PLR, platelets and ypTNM in the study population

| Patient characteristic | Overall survival | Disease-free survival |
|------------------------|-----------------|-----------------------|
|                        | Exp(b) 95% CI p-value | Exp(b) 95% CI p-value |
| Adjuvant chemotherapy: |                 |                       |
| no                     | 1.92 0.68-5.43 0.22 |                       |
| yes                    |                 |                       |
| Stage:                 |                 |                       |
| 3                      | 1.89 0.21-17 0.57 |                       |
| 4                      |                 |                       |
| NLR:                   |                 |                       |
| <3.27                  | 4.22 0.99-17.97 0.053 | 3.49 1.19-10.24 0.024* |
| >3.27                  |                 |                       |
| Platelets:             |                 |                       |
| <300                   | 0.62 0.22-1.72 0.36 |                       |
| >300                   |                 |                       |
| PLR:                   |                 |                       |
| <150                   | 2.14 0.61-7.56 0.24 |                       |
| >150                   |                 |                       |
| ypTNM staging:         |                 |                       |
| ypT0-2 N0              | 5.02 0.6-41.6 0.14 | 10.66 2.3-49.49 <0.0026* |
| ypT3-4/N+              |                 |                       |

NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; Exp(b) = exponential; 95% CI = 95% confidence interval; *p value lower than 0.05 set as the level of significance
with tumor response after neoadjuvant therapy\textsuperscript{31}. All these findings are in concordance with our results as well. Giakoustidis et al.\textsuperscript{12} analyzed 169 CRC patients that underwent neoadjuvant CRT; 42\% of the study patients had increased NLR > 2.5, which correlated with shorter OS but not with DFS. This finding is partially concordant with our results.

Kim et al.\textsuperscript{13} report that patients with pre-treatment thrombocytosis had decreased 3-year DFS and OS when compared with patients with normal pre-treatment platelet counts. Therefore, the same authors conclude that thrombocytosis is a negative indicator in these patients, which points to shorter survival in patients with CRC\textsuperscript{13}. Wei et al.\textsuperscript{14} showed that the baseline platelet count correlated with recurrence in 286 patients with stage II-III CRC receiving adjuvant chemotherapy. The optimal cut-off affecting recurrence was 276\times 10^9/L. Kaplan-Meier showed that those with baseline platelet count > 276\times 10^9/L receiving adjuvant chemotherapy had worse DFS than those with baseline platelet count \leq 276\times 10^9/L, with 5-year DFS 66\% and 80\%, respectively. At the end, CRC patients receiving adjuvant chemotherapy with baseline platelet count > 276\times 10^9/L had worse prognosis\textsuperscript{14}. The same correlation between thrombocytosis and DFS but not OS was recorded in our patients.

The results of this study showed that, irrespective of the statistical method employed (except for multivariate analysis), NLR was a significant predictor of DFS and OS. It may be that the patient follow up period was too short, so the results of multivariate analysis did not reach significance regarding NLR and OS (patients were recruited from the 2011-2015 period). However, the results almost reached significance (p=0.053).

The neutrophil-to-lymphocyte ratio is not a predictor of pathological response to neoadjuvant CRT, i.e. be the NLR high or low, we could not conclude whether the patient would have better or worse response to neoadjuvant CRT, which is consistent with other authors. However, the results of univariate analysis showed significant correlation of advanced pathological TNM stage with both DFS and OS.

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Kronična upala je povezana s mnogim karcinomima. Čini se da lako dostupni i uobičajeni upalni biljezi u serumu mogu biti prognostički čimbenik za ukupno preživljenje i razdoblje bez bolesti u bolesnika s različitim karcinomima. Prijeoperacijski odnos neutrofil a i limfocita (NLR) te trombocita i limfocita (TLR), kao i hemoglobinemija, trombocitoza, neutropenija i leukocitoza su povezani s ukupnim preživljenjem i razdobljem bez bolesti u bolesnika s kolorektalnim karcinomom (KRK), doduše, s proturječnim rezultatima. Kompletna krvna slika, NLR i TLR su određeni u 71 bolesnika s KRK (stadij 3 i 4) nakon neoadjuvantne kemoradioterapije i prije kirurškog zahvata, koji su liječeni na Klinici za tumore u Zagrebu. Statistička analiza je uključivala Mann-Whitneyev U test, Studentov t-test, univarijatnu i multivarijatnu analizu. Rezultati Mann-Whitneyeva U testa i Studentova t-testa su pokazali da su broj neutrofil a (p=0,024), NLR (p=0,003) i TLR (p=0,007) značajno povezani s ukupnim preživljenjem. Ipak, nije bilo značajne povezanosti između dobi, broja leukocita, limfocita i trombocita te hemoglobina s ukupnim preživljenjem bolesnika. Nadalje, isti testovi su pokazali da su broj leukocita (p=0,04), neutrofila (p=0,0014) i trombocita (p=0,006), NLR (p=0,0006) i TLR (p=0,0015), kao i hemoglobin (p=0,028) značajno povezani s razdobljem bez bolesti. Rezultati univarijatne analize su pokazali da, za razliku od TLR, NLR korelira s ukupnim preživljenjem i preživljenjem bez bolesti. Rezultati multivarijatne analize su pokazali kako NLR (granična vrijednost 3,27) i uznapiševale patološki stadij TNM značajno koreliraju s preživljenjem bez bolesti za razliku od ukupnog preživljenja. Čini se da je NLR pouzdan biljeg ukupnog preživljenja kao i preživljenja bez bolesti u bolesnika s KRK nakon neoadjuvantne kemoradioterapije i prije kirurškog zahvata.

Ključne riječi: Upala; Krvna slika; Kolorektalni tumori; Preživljenje bez bolesti; C-reaktivni protein

Kronična upala je povezana s mnogim karcinomima. Čini se da lako dostupni i uobičajeni upalni biljezi u serumu mogu biti prognostički čimbenik za ukupno preživljenje i razdoblje bez bolesti u bolesnika s različitim karcinomima. Prijeoperacijski odnos neutrofil a i limfocita (NLR) te trombocita i limfocita (TLR), kao i hemoglobinemija, trombocitoza, neutropenija i leukocitoza su povezani s ukupnim preživljenjem i razdobljem bez bolesti u bolesnika s kolorektalnim karcinomom (KRK), doduše, s proturječnim rezultatima. Kompletna krvna slika, NLR i TLR su određeni u 71 bolesnika s KRK (stadij 3 i 4) nakon neoadjuvantne kemoradioterapije i prije kirurškog zahvata, koji su liječeni na Klinici za tumore u Zagrebu. Statistička analiza je uključivala Mann-Whitneyev U test, Studentov t-test, univarijatnu i multivarijatnu analizu. Rezultati Mann-Whitneyeva U testa i Studentova t-testa su pokazali da su broj neutrofila (p=0,024), NLR (p=0,003) i TLR (p=0,007) značajno povezani s ukupnim preživljenjem. Ipak, nije bilo značajne povezanosti između dobi, broja leukocita, limfocita i trombocita te hemoglobina s ukupnim preživljenjem bolesnika. Nadalje, isti testovi su pokazali da su broj leukocita (p=0,04), neutrofila (p=0,0014) i trombocita (p=0,006), NLR (p=0,0006) i TLR (p=0,0015), kao i hemoglobin (p=0,028) značajno povezani s razdobljem bez bolesti. Rezultati univarijatne analize su pokazali da, za razliku od TLR, NLR korelira s ukupnim preživljenjem i preživljenjem bez bolesti, iako je korelacija zamalo postignuta između TLR i razdoblja bez bolesti (p=0,059). Nije bilo povezanosti između bolesnikove bolesti i spola, stadijia tumora i neoadjuvantne kemoradioterapije s ukupnim preživljenjem i razdobljem bez bolesti. Rezultati multivarijatne analize su pokazali kako NLR (granična vrijednost 3,27) i uznapiševale patološki stadij TNM značajno koreliraju s preživljenjem bez bolesti za razliku od ukupnog preživljenja. Čini se da je NLR pouzdan biljeg ukupnog preživljenja kao i preživljenja bez bolesti u bolesnika s KRK nakon neoadjuvantne kemoradioterapije i prije kirurškog zahvata.

Ključne riječi: Upala; Krvna slika; Kolorektalni tumori; Preživljenje bez bolesti; C-reaktivni protein