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ORIGINAL ARTICLE

Neutrophils-to-lymphocytes, lymphocytes to-monocytes and platelets-to-lymphocytes ratios - predictive biomarkers for response to neoadjuvant chemotherapy in breast cancer

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Summary

Purpose: *Several biomarkers have been reported to correlate with neoadjuvant chemotherapy response. Our aim was to establish the correlation between neutrophils-to-lymphocytes (NLR), lymphocytes-to-monocytes (LMR), and platelets-to-lymphocytes ratios (PLR) and the Miller Payne grade (MPG) and Residual Cancer Burden Score (RCB), as indicators to response to chemotherapy.*

Methods: *Data were retrospectively collected from the First Surgical Clinic database between January 2016 and December 2018.*

Results: *96 patients were included in the study. The multivariate regression analysis showed a statistical correlation between oestrogen (ER) and progesterone receptor (PR) status, Ki67 over 15%, and tumour infiltrating lymphocytes (TILs) and MPG and RCB. For the three studied ratios, p value was statistical not significative. ROC curve showed a cut-off value of 2.7 NLR, for which correlation with the pathological complete response to chemotherapy (pCR) was significative (p=0.03).*

Conclusions: *Our findings suggest that NLR can be a predictive biomarker for pCR. Further studies, on larger sample size, are necessary to establish the correlation with MPG and RCB.*

Key words: *biomarker, breast cancer, Miller Payne grade, neoadjuvant chemotherapy, pCR, residual cancer burden*

Introduction

As the incidence of breast cancer continues to remain elevated, it is important to study and understand the immunological response in the development and progression of this disease. Neoadjuvant chemotherapy is the standard treatment in locally advanced tumours. Chemo-resistance represents a crucial problem of breast cancer. The chemo-responsiveness of the tumour is determined by patient-related factors as well as by intrinsic tumour characteristics [1,2].

Many studies tried to establish the correlation between immunological markers and the response to neoadjuvant chemotherapy [3-4]. Moreover, pathological complete response (pCR) to chemotherapy is associated with lower recurrence rates and better overall survival [5].

Many biomarkers were proposed as predictive. NLR, LMR and PLR were among the most studied, the results showing a strong correlation between the three ratios with pCR and survival.

However, since the outcome of patients can be influenced not only by a pCR, the objective of our study was to determine their correlation with the most known parameters for evaluating the response to neoadjuvant chemotherapy, the Miller Payne grade (MPG) and the Residual Cancer Burden Score (RCB).

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Methods

Study cohort

We identified all patients with locally advanced breast cancer that received neoadjuvant chemotherapy and subsequent breast surgery at the First Surgical Clinic, Cluj-Napoca, Romania, between January 2016 and December 2018. We excluded all patients with stage IV disease or inflammatory breast cancer, and also patients without available pathology reports and laboratory test results. Data of 96 patients were analyzed. From the electronic medical records, we extracted information about age, menopausal status, tumour characteristics (stage and grade, intrinsic molecular subtype, histopathological features and TILs), lymph node status, chemotherapy regimen, type of surgical intervention, and laboratory data (absolute number of neutrophils, lymphocytes, monocytes, and platelets - all values from blood samples taken before the initiation of chemotherapy).

Table 1. Characteristics of patients included in the study and their correlation with Miller Payne grade and Residual Cancer Burden score

Characteristics n=96	*n (%)*	*Miller Payne grade* p	*RCB score* p
Age at diagnosis (years), median (range)	54.93 (27-76)	0.19	0.5
Menopausal status		0.35	0.77
Premenopausal	24 (25)		
Postmenopausal	72 (75)		
Tumour size		0.04	0.01
T1	8 (8.33)		
T2	42 (43.75)		
T3	25 (26.04)		
T4	21 (21.87)		
Lymph nodes status		0.04	0.03
N0	3 (3.12)		
N1	65 (67.70)		
N2	25 (26.04)		
N3	3 (3.12)		
Histological subtype		0.07	0.21
Ductal	88 (91.66)		
Lobular	3 (3.12)		
Other	5 (5.20)		
Tumour grade		0.07	0.01
G1	20 (20.83)		
G2	48 (50)		
G3	28 (29.16)		
Estrogen receptor positivity	62 (64.58)	<0.05	<0.05
Progesteron receptor positivity	52 (54.16)	<0.05	<0.05
HER-2 receptor positivity	29 (30.20)	0.36	0.73
Ki67 >15%	60 (62.50)	0.02	<0.05
Intrinsic subtype (IHC 4)		0.01	<0.05
Luminal A	23 (23.95)		
Luminal B	39 (40.62)		
HER-2 enriched	9 (9.37)		
Triple negative	25 (26.04)		
TILs		0.01	<0.05
0	3 (3.12)		
1	31 (32,29)		
2	48 (50)		
3	14 (14,58)		
NLR, median (range)	2.70 (0.63-6.88)	0.17	0.82
LMR, median (range)	2.70 (0.59-8.24)	0.35	0.90
PLR, median (range)	28.71 (6.28-104)	0.56	0.93

For TILs, the Klintrup score was used. Therefore, the patients were scored with 0 for absent inflammatory cells, 1 for mild and patchy inflammatory cells, 2 for prominent band-like inflammatory cells, and 3 for rich, cup-like inflammatory cells. All data were taken from the pre-treatment pathology reports.

For each patient we calculated NLR as the ratio between neutrophils and lymphocytes values, LMR as the ratio between lymphocytes and monocytes values, and PLR as the ratio between platelets and lymphocytes values.

Table 1 summarises the characteristics of the studied population.

Treatment

The neoadjuvant chemotherapy regimen consisted of 4 cycles of doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2), followed by 4 cycles of docetaxel (75 mg/m^2). Some patients received doxorubicin (60 mg/m^2) plus docetaxel (75 mg/m^2) or doxorubicin (60 mg/m^2) plus cyclophosphamide (600 mg/m^2) by intravenous infusion every 3 weeks for 6 cycles. After 4-6 weeks the patients were admitted for surgical treatment: either breast-conserving surgery, or mastectomy with axillary lymph node dissection (ALND).

Histopathological response assessment

The histopathological response was evaluated on the surgical excision sample by Miller-Payne grade and Residual Cancer Burden score.

Statistics

The statistical analysis was performed using Epi-Info version 7.2.2.6.

We evaluated the correlation between age, menopausal status, tumour size pre-chemotherapy, lymph nodes status, histological subtype, tumour grade, receptors status, intrinsic subtype, and TILs with the MPG and RCB using Cox multivariate regression analysis.

For NLR, LMR and PLR we analyzed the correlation with the MPG and RCB score using linear regression. To establish the capacity of NLR, LMR and PLR on predicting a pCR we calculated the cut-off values using the receiver operating characteristics (ROC) curve. For each ratio sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) were calculated. The relation between cut-off values and pCR was evaluated with Fisher test. A p value under 0.05 was considered statistically significant.

Results

The 96 patients included in the study had a mean age at the time of the treatment of 54.93 years, 75% of them being postmenopausal. Of all patients 96.87% presented with lymph node infiltration (N1-N3). The majority of the cases were ductal carcinomas (91.66%) and luminal B (40.62%). Table 1 presents the histological and immunohistological characteristics of the tumours.

Regarding neoadjuvant chemotherapy regimens, we used anthracycline-based therapy in 26 (27.08%) cases, taxanes-based therapy in 21 (21.87%) cases and combined anthracycline and taxanes therapy in 49 (51.04%) cases. No significant statistical correlation was determined between chemotherapy regimen and MPG and RCB ($p>0.05$). pCR was obtained in 25 (26.04%) cases. 35 (36.45%) of the patients underwent breast-conserving therapy and ALND and 61 (63.54%) underwent modified radical mastectomy.

The linear regression analysis showed a significant statistical correlation between tumour size, lymph node status and MPG and RCB ($p<0.05$). For both scores we determined a significant correlation with ER and PR positivity (Table 1), ki67 over 15% (Table 1), and with the intrinsic subtype ($p=0.01$ for MPG and $p<0.05$ for RCB), triple-negative tumours having the worst response to neoadjuvant chemotherapy. Another factor that determined the response to neoadjuvant chemotherapy was TILs. We obtained a p value <0.05 for both MPG and RCB (Table 1).

No statistically significant correlation was obtained between NLR, LMR, PLT and MPG, and RCB ($p>0.05$, Table 1) on linear regression analysis.

ROC curve analysis suggested that the optimum cut-off point of NLR, LMR, and PLR for obtaining pCR were 2.7 (95% CI, estimated standard error 0.06, $p=0.03$ Fisher Test), 2.1 (95% CI, estimated standard error 0.05, $p=0.35$ Fisher Test), and 21.82 (95% CI, estimated standard error 0.06, $p=0.81$ Fisher Test). Table 2 shows the accuracy, specificity, sensitivity, PPV, and NPV calculated for the cut-off points.

Table 2. Cut off points analysis for NLR, LMR, and PLR

	Accuracy (%)	*Specificity (%)*	*Sensitivity (%)*	*PPV (%)*	*NPV (%)*
NLR (2.7)	58.33	53.52	72.00	35.29	84.44
LMR (2.1)	44.74	23.53	88.00	36.07	80.00
PLR (21.82)	51.04	49.30	56.00	28.00	76.09

PPV: positive predictive value, NPV: negative predicted value. For other abbreviations see text.

Discussion

This study showed no significative relationship between NLR, LMR, and PLR and MPG or RCB. However, the analysis between the cut-off point value of NLR (2.7) determined by ROC curve analysis and the pCR showed a significative correlation between increased NLR (over 2.7) and pCR to neoadjuvant chemotherapy. Also the obtained NPV and sensitivity values were considerable high. We couldn't find any significant correlation between LMR or PLR and pCR ($p>0.05$).

Neutrophils, lymphocytes, monocytes and platelets have different inflammatory roles. Neutrophils increase the turnover proliferation, favouring invasion and secreting factors that promote tumour growth. Monocytes differentiate into tumour-associated macrophages, promoting proliferation, invasion, metastasis, neovascularisation, and recurrence. Platelets release factors that activate angiogenesis, stimulating tumour progression. Lymphocytes, especially cytotoxic T cells, have an antitumor immune response, stimulating apoptosis of tumor cells and suppressing their growth [6-8]. These functions motivate the study of NLR, LMR, and PLR as prognostic factors to tumor response in neoadjuvant chemotherapy.

Many studies tried to determine the correlation between the 3 ratios with pCR to neoadjuvant chemotherapy and long term survival.

For NLR, increased values prior to neoadjuvant chemotherapy were associated with lower rates of pCR [9,10]. No cut-off value was determined, with studies showing that either a value over 3.33 [11], or over 2.05 [12,13] can be considered statistically significant. In our study the cut-off point value for which we obtained statistically significant correlation was 2.7. In 2018 Duan et al published a meta-analysis on 21 studies that demonstrated that NLR can be considered a predictive biomarker for overall survival prognosis in patients with breast cancer [14], in relation with other prior published results [15,16].

We couldn't find a significant statistical correlation between LMR and response to neoadjuvant chemotherapy, although we obtained high values for sensitivity (88%) and NPV (80%) for a cut-off point value calculated at 2.1. Some authors published significant results for values higher to 5.2 regarding both chemotherapy response and survival [17] while others reported that a LMR over 4.7 can be significantly associated with a better outcome in early stage breast cancer [18].

Studies comparing NLR and LMR as predictive factors for chemotherapy response and survival showed better correlations for NLR [19].

PLR was proposed as a predictive and prognostic biomarker, especially for triple-negative breast cancers [20,21]. The cut-off values proposed by other studies vary between 138.19 and 292 [22-24]. However, our study determined a cut-off value of 21.82 and no significant correlation was obtained.

We showed a significant correlation between ER and PR, and proliferation rate described by ki67 and neoadjuvant chemotherapy response evaluated by MPG and RCB. To our knowledge, this is the first study to consider MPG and RCB in the study of factors predictive for chemotherapy response. Although the importance of TILs for pCR is recognised [25], we managed to demonstrate the significant correlation between TILs and MPG and RCB ($p<0.05$), showing that a high Klintrup score is associated with a better response to chemotherapy.

Despite pCR is associated with a better outcome and overall survival [26,27], we consider that a stratified analysis according to MPG and RCB needs to be done. Preliminary published results show a different outcome according to RCB score [28].

Our study has some limitations. First, it is a single-centre retrospective study. Many data were unavailable and this resulted in a small sample size. Second, the neoadjuvant therapy was not standardised and because of small sample size we couldn't perform an analysis differentiating the three specific regimens. Third, NLR, LMR and PLR can be influenced by various comorbidities, data that we didn't include in our analysis. Therefore, the results that we obtained can be biased by these three limitations of our study.

Further studies with larger sample size are necessary to establish the relationship between the three biomarkers, NLR, LMR, and PLR, and the response to neoadjuvant chemotherapy evaluated by MPG and RCB. Also TILs can be considered as a predictive biomarker due to its correlation with MPG and RCB.

Conflict of interests

The authors declare no conflict of interests.

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