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Combination of peripheral neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio is predictive of pathological complete response after neoadjuvant chemotherapy in breast cancer patients

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ABSTRACT

The immune system seems to play a fundamental role in breast cancer responsiveness to chemotherapy. We investigated two peripheral indicators of immunity/inflammation, i.e. neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR), in order to reveal a possible relationship with pathological complete response (pCR) in patients with early or locally advanced breast cancer treated with neoadjuvant chemotherapy (NACT).

We retrospectively analyzed 373 consecutive patients affected by breast cancer and candidates to NACT. The complete blood cell count before starting NACT was evaluated to calculate NLR and PLR. ROC curve analysis determined threshold values of 2.42 and 104.47 as best cut-off values for NLR and PLR, respectively. The relationships between NLR/PLR and pCR, along with other clinical-pathological characteristics, were evaluated by Pearson's χ 2 or Fisher's exact test as appropriate. Univariate and multivariate analyses were performed using a logistic regression model.

NLR and PLR were not significantly associated with pCR if analyzed separately. However, when combining NLR and PLR, patients with a NLR^{low}/PLR^{low} profile achieved a significantly higher rate of pCR compared to those with NLR^{high} and/or PLR^{high} (OR 2.29, 95% CI 1.22–4.27, p 0.009). Importantly, the predictive value of NLR^{low}/PLR^{low} was independent from common prognostic factors such as grading, Ki67, and molecular subtypes.

The combination of NLR and PLR may reflect patients' immunogenic phenotype. Low levels of both NLR and PLR may thus indicate a status of immune system activation that may predict pCR in breast cancer patients treated with NACT.

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1. Introduction

Breast cancer is the most common female neoplasm and the second cause of cancer mortality in women in industrialized countries [1,2].

Neoadjuvant chemotherapy (NACT) is widely used to permit surgery in locally advanced and inflammatory breast cancer or to downstage tumor in order to achieve breast-conserving surgery [3–5]. It has been proposed that pathological complete response (pCR) after NACT could be considered a good surrogate marker of disease free survival (DFS) and overall survival (OS), particularly in

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patients with more aggressive subtypes, i.e. triple-negative or HER2-positive breast cancer [6,7]. However, this does not apply to hormone receptor (HR)-positive, HER2-negative luminal subtypes, since pCR is rarely observed for these tumors and patients maintain a good long-term prognosis independently of pCR [6,8–10]. Apart from the molecular subtypes, thus far, no other biomarker has been validated as predictive factor for pCR or prognostic factor for DFS and/or OS after NACT, including Ki67 and Residual Cancer Burden [11,12].

Recently, the immune system has been recognized to play a pivotal role in the responsiveness of breast cancer to chemotherapy [13,14]. Although breast cancer is not a high neoantigens producers [15], it is frequently infiltrated by lymphocytes (tumor infiltrating lymphocytes, TILs), which may largely vary across the molecular subtypes [16,17]. The presence of TILs in breast cancer strongly correlates with pCR after NACT [18–20]. Distinct tumor-infiltrating cell subtypes, such as regulatory T lymphocytes (Treg) and myeloid-derived-suppressor cells (MDSC), lead to immune suppression, and were shown to reduce the efficacy of NACT in breast cancer [21]. Consistent with such a role of the immune system, breast cancer shows a propensity to respond to immune checkpoint inhibitors (ICIs), albeit to a lesser degree of efficacy compared to other neoplasms such as melanoma, kidney and lung cancer [22].

Thus, given the clear influence of the tumor immune microenvironment on breast cancer response to therapy, several lines of research are being carried out to define the role of peripheral immune system on the outcome of breast cancer, and particularly on the response to NACT [23].

Peripheral indicators of immunity/inflammation, including Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Platelet Mean Volume, and White Blood Cells-to-Lymphocyte Ratio, have been extensively studied for possible correlations with pCR. Overall, low ratio of these parameters indicates a systemic background of reduced inflammation and immune system activation, resulting in better response to therapy [24–33]. However, the contribution of these immunity/inflammation indicators to NACT response has not been analyzed along with other predictive response factors, such as molecular subtypes, grading, and Ki67.

In the study herein presented, we explored if basal NLR and/or PLR were able to identify patients affected by breast cancer who were more likely to achieve pCR after NACT and if the predictive value of these biomarkers was independent from other clinical/pathological factors.

2. Patients and materials

2.1. Patients

Patients with early or locally advanced breast cancer who were candidates to NACT and referred to the Medical Oncology Unit of the S.S. Annunziata Hospital of Chieti from January 1st, 1999 through May 31st, 2018 were consecutively screened for participation in the study.

All diagnoses were confirmed histologically by biopsy and all patients received standard NACT chemotherapy. The most common regimens included anthracycline and taxanes. Other regimens included: EC (epirubicin and cyclophosphamide), FEC (fuorouracil, epirubicin and cyclophosphamide); CMF (fuorouracil, methotrexate and cyclophosphamide); single-agent epirubicin; E-CMF (single-agent epirubicin followed by CMF); single-agent taxanes; and other combinations with platinum compounds, vinorelbine and pegylated doxorubicin. Neoadjuvant Trastuzumab was administered to patients whose tumors were HER2-positive, except for those who have undergone NACT before 2005, the year when trastuzumab was approved in Italy. Surgical procedures consisted of mastectomy or breast-conserving surgery (BCS) and axillary lymph node dissection or sentinel lymph node biopsy as clinically indicated.

Clinical and pathological tumor staging were defined according to the 8th edition of the American Joint Committee Cancer Staging Manual. The local institutional review board approved the project. This study was conducted in accordance with the Declaration of Helsinki. Written informed consents were secured from all the participants. This study adheres to the REMARK guidelines [34].

2.2. Pathological assessments

All breast cancer biopsies and surgical specimens were processed for immunohistochemistry (IHC). Tumors were considered ER or PR positive if $\geq$ 10% of cells had nuclear receptor staining [35]. Ki-67, detected by MIB-1 antibody [36], was set at a cutoff point of 14% to discriminate between luminal A and luminal B tumors [37]. The nuclear grade was assessed according to the Nottingham grading system [38]. HER2 positivity was defined according to the ASCO/CaP guidelines, i.e. a score 3 + in ICH by HercepTest™ (Dako Italia, Milan, Italy) and/or a positive HER2 gene amplification by FISH or SISH [39]. Tumor molecular subtypes were classified as Luminal A, Luminal B/HER2-negative, Luminal B/HER2-positive, HER2-enriched and Triple-negative, as already described [37].

2.3. Blood samples and data collection

Peripheral complete blood count was performed at baseline, i.e. just before starting NACT. NLR was provided by the ratio between the absolute count of neutrophils and the absolute count of lymphocytes. PLR was calculated by dividing the absolute number of platelets by the absolute number of lymphocytes. All blood cell assessments were centrally performed in our institutional laboratory according to the standardized operative procedures.

Data concerning the clinical and pathological features of all patients, along with the type of treatment administered and related outcome, were retrospectively collected and entered into an anonymized dedicated database.

2.4. Study endpoint

The main objective of the study was to verify the possible predictive value of NLR and/or PLR for pathologic complete response (pCR). We defined pCR as the absence of invasive breast cancer in the breast and axillary lymph nodes in the surgical specimen after NACT (ypT0/ypTis, ypN0). Noninvasive breast residuals (ductal carcinoma-in-situ) were allowed [40].

2.5. Statistical analysis

Since no validated cut-off value is reported in the literature, NLR and PLR cut-off points were calculated considering the maximum (sensitivity + specificity) point of the Receiver Operating Characteristic (ROC) curve for the prediction of pCR.

The relationships between NLR/PLR, pCR and other key clinical-pathological characteristics were evaluated by Pearson's χ^2 or Fisher's exact test as appropriate. Univariate and multivariate analyses were performed using a logistic regression model. Odds Ratio (OR) was reported with the corresponding 95% confidence intervals (95% CI). A $p < 0.05$ was considered statistically significant.

Statistical analyses were performed using the SPSS Statistic software 19 (SPSS Inc., Chicago, III).

3. Results

3.1. Patient and tumor baseline characteristics

We identified 388 consecutive breast cancer patients treated with NACT. Among them, 373 patients had pre-treatment complete blood count and were included in the study. Patients' baseline characteristics are shown in Table 1. Median age at diagnosis was 50 years (range 26–82). Prevalent histology was invasive ductal carcinoma (77.0%) and the majority of cases was cT2 in tumor size at the diagnosis (66.8%). Only 26.8% of tumors were well differentiated (G1) and high Ki67 proliferation index (≥14) was expressed in 53.3% of cases. One third of patients (35.4%) had a Luminal A tumor subtype, 11.8% Luminal B/HER2-negative, 18.4% Luminal B/HER2-positive, 17.7% HER2-enriched and 16.6% triple-negative.

Two hundred and twelve patients (56.8%) received a classical anthracycline- and taxane-based sequential chemotherapy, and 104 received trastuzumab in combination with chemotherapy. Most patients (95.6%) received at least 6 cycles of chemotherapy. After NACT, 237 patients (63.5%) underwent a conservative surgical approach, while the remaining 136 (36.5%) were treated by mastectomy.

3.2. Relationship between baseline characteristics and NLR/PLR

The NLR cut-off point chosen according to the ROC curve analysis was 2.42. This value allowed identifying two populations: NLR^{low} (≤2.42), 268 patients (71.8%) and NLR^{high} (>2.42), 105 patients (28.2%). Similarly, a cut-off value of 104.47 was identified by the ROC curve for PLR and two populations of patients were identified: PLR^{low} (≤104.47), 93 patients (34.1%) and PLR^{high} (>104.47), 280 patients (63.9%). The NLR and PLR distribution according to the previously defined cut-offs is reported in Table 1. At univariate analysis, older women (>50 years) showed higher probability to be NLR^{low} ($p < 0.001$) and patients with advanced stage of disease (cT3-4) were more frequently PLR^{high} ($p = 0.025$). None of the other baseline characteristics was statistically associated with either NLR or PLR.

3.3. Relationship between baseline characteristics and pCR

Ninety-one patients (24%) obtained a pCR after NACT. Classical breast cancer poor prognostic factor, such as high grade, ki67 > 14, HR negativity, and HER2 positivity, were all associated with pCR in univariate analysis (Table 2). In particular, the probability to achieve pCR was 4-fold higher in HR-negative compared to HR-positive tumors (OR 4.37, 95% CI 2.70–7.19, $p < 0.001$), and more than double in HER2-positive compared to HER2-negative tumors (OR 2.66, 95% CI 1.64–4.31, $p < 0.001$). Similarly, higher rates of pCR were observed in patients with high grade (G2 or G3) or Ki67 ≥ 14 tumors compared to those with low grade or Ki67 < 14 tumors (OR 2.19, 95% CI 1.48–3.25, $p < 0.001$ and OR 2.66, 95% CI 1.64–4.31, $p < 0.001$, respectively). Consistently, luminal B or Triple-negative or HER2-enriched subtypes had significantly higher rates of pCR compared to luminal A. Neither NLR^{low} nor PLR^{low} were able to predict pCR in the entire population. However, among the 14 patients (10.6%) with luminal A tumor who obtained pCR, 12 (85.7%) were NLR^{low} and 2 (24.3%) were NLR^{high}, although such difference

Table 1
Association of baseline characteristics to NLR or PLR.

Variable	n (%) (n = 373)	NLR			PLR		
		Low (%) (n = 268)	High (%) (n = 105)	p value*	Low (%) (n = 93)	High (%) (n = 280)	p value
Age (years)				**0.000**			0.077
≤50	192 (51.5)	123 (64.1)	69 (35.9)		40 (20.8)	152 (79.2)	
>50	181 (48.5)	145 (80.1)	36 (19.9)		53 (29.3)	128 (70.7)	
Histologic type				0.832			0.178
Ductal	287 (76.9)	207 (72.1)	80 (27.9)		69 (24.0)	218 (76.0)	
Lobular	32 (8.60)	24 (75.0)	8 (25.0)		12 (37.5)	20 (62.5)	
Others	54 (14.5)	37 (68.5)	17 (31.5)		14 (25.9)	40 (74.1)	
Grade				0.294			0.233
G1	100 (26.8)	70 (70.0)	30 (30.0)		30 (30.0)	70 (70.0)	
G2	188 (50.4)	141 (75.0)	47 (25.0)		43 (22.9)	145 (77.1)	
G3	55 (14.7)	34 (61.8)	21 (38.2)		10 (18.2)	45 (81.8)	
Unknown[a]	30 (8.00)	21 (70.0)	9 (30.0)		10 (33.3)	20 (66.7)	
Stage				0.118			**0.025**
cT1	40 (10.7)	28 (70.0)	12 (30.0)		7 (17.5)	33 (82.5)	
cT2	249 (66.8)	188 (75.5)	61 (24.5)		74 (29.7)	175 (70.3)	
cT3	70 (18.8)	43 (61.4)	27 (38.6)		10 (14.3)	60 (85.7)	
cT4	14 (3.70)	9 (64.3)	5 (35.7)		2 (14.3)	12 (85.7)	
Ki-67				0.697			0.753
<14%	100 (26.8)	71 (71.0)	29 (29.0)		25 (25.0)	75 (75.0)	
≥14%	199 (53.4)	148 (74.4)	51 (25.6)		45 (22.6)	154 (77.4)	
Unknown[a]	74 (19.8)	49 (66.2)	25 (33.8)		18 (24.3)	56 (75.7)	
Hormone Receptor (HR)				0.549			0.917
Positive	245 (65.7)	179 (73.1)	66 (26.9)		62 (25.3)	183 (74.7)	
Negative	128 (34.3)	89 (69.5)	39 (30.5)		31 (24.2)	97 (75.8)	
HER2				0.401			0.198
Positive	238 (63.7)	167 (70.2)	71 (29.8)		65 (27.3)	173 (72.7)	
Negative	135 (36.3)	101 (70.4)	34 (25.2)		28 (20.7)	107 (79.3)	
Molecular subtype				0.554			0.507
Luminal A	132 (35.4)	93 (70.5)	39 (29.5)		38 (28.8)	94 (71.2)	
Luminal B/HER2-	44 (11.8)	34 (77.3)	10 (22.7)		12 (27.3)	32 (72.7)	
Luminal B/HER2+	69 (18.5)	52 (74.4)	17 (24.6)		12 (17.4)	57 (82.6)	
Triple Negative	62 (16.6)	40 (64.5)	22 (35.5)		15 (24.2)	47 (75.8)	
HER2 enriched	66 (17.7)	49 (74.2)	17 (25.8)		16 (24.2)	50 (75.8)	

* Significant values are indicated in bold.
[a] Unknown not included in the analysis.

Table 2
Association of patient/tumor characteristics to pCR in univariate analysis.

Variable	n (%) (n = 373)	pCR (%) (n = 91)	Odds ratio	95% CI	p value*
Age (years)					
≤50	192 (51.5)	43 (22.4)	1.00		
>50	181 (48.5)	48 (26.6)	1.25	0.78–2.00	0.354
Histologic type					
Others	54 (14.4)	8 (14.8)	1.00		
Ductal	287 (76.9)	75 (26.1)	2.03	0.92–4.50	0.063
Lobular	32 (8.6)	8 (25.0)	1.38	0.80–2.40	0.247
Grade					
G1	100 (26.8)	11 (11.0)	1.00		
G2	188 (50.4)	58 (30.9)	2.69	1.39–5.23	**0.003**
G3	55 (14.7)	19 (34.5)	2.19	1.48–3.25	**<0.001**
Unknown[a]	30 (8.00)	3 (10.0)			
Stage					
cT1/cT2	289 (77.5)	67 (23.2)	1.00		
cT3/cT4	84 (22.5)	24 (28.6)	1.13	0.65–1.97	0.665
Ki-67					
<14%	100 (26.8)	16 (16.0)	1.00		
≥14%	199 (53.3)	61 (30.7)	2.32	1.26–4.29	**0.004**
Unknown[a]	74 (19.8)	14 (18.9)			
Hormone Receptor (HR)					
Positive	245 (65.7)	36 (14.7)	1.00		
Negative	128 (34.3)	55 (43.0)	4.37	2.70–7.19	**<0.001**
HER2					
Positive	238 (63.8)	42 (17.6)	1.00		
Negative	135 (36.2)	49 (36.3)	2.66	1.64–4.31	<0.001
Molecular subtype					
Luminal A	132 (35.4)	14 (10.6)	1.00		
Luminal B/HER2-	44 (11.8)	7 (15.9)	1.59	0.60–4.25	0.362
Luminal B/HER2+	69 (18.5)	15 (21.7)	1.53	1.03–2.28	**0.036**
Triple Negative	62 (16.6)	21 (33.9)	1.62	1.26–2.10	**<0.001**
HER2 enriched	66 (17.7)	34 (51.5)	1.73	1.44–2.07	**<0.001**
Chemotherapy regimen					
Various	57 (15.3)	8 (14.0)	1.00		
Antracycline and Taxane	212 (56.8)	40 (18.9)	1.19	0.79–1.80	0.387
Chemio + Trastuzumab	104 (27.9)	43 (41.3)	4.32	1.89–10.0	**<0.001**
NLR					
High	105 (28.2)	20 (19.0)	1.00		
Low	268 (71.8)	71 (26.5)	1.53	0.88–2.67	0.125
PLR					
High	280 (75.1)	62 (22.1)	1.00		
Low	93 (25.9)	29 (31.2)	1.59	0.95–2.68	0.084
NLR/PLR					
High/High	100 (26.8)	19 (19.0)	1.00		
Low/High	180 (48.3)	43 (23.9)	1.33	0.73–2.45	0.346
High/Low	5 (1.3)	1 (20.0)	1.03	0.33–3.17	0.956
Low/Low	88 (23.6)	28 (31.8)	1.98	1.01–3.89	**0.044**
Surgery					
Mastectomy	136 (36.5)	30 (22.1)	1.00		
Breast-conserving surgery	237 (63.5)	61 (25.7)	1.23	0.74–2.08	0.426

* Significant values are indicated in bold.
[a] Unknown not included in the analysis.

did not fully reach the preset threshold for statistical significance (p = 0.051) (data available upon request).

Assuming that the balance between inflammation and immunity would be better described by the combination of NLR and PLR, i.e. a systemic pro-immunogenic phenotype displaying both the indicators low (NLR^{low}/PLR^{low}) and a pro-inflammatory phenotype showing at least one of the two indicators high (NLR^{high} and/or PLR^{high}), we analyzed the predictive value of NLR/PLR combination for pCR. Consistently, we found the highest rate of pCR (32%) in the group of patients with a NLR^{low}/PLR^{low} profile and the lowest rate (19%) in the group with a NLR^{high}/PLR^{high} profile. In more detail, NLR^{low}/PLR^{low} patients had twice the chance to achieve a pCR compared to NLR^{high}/PLR^{high} patients (OR 1.98, 95% CI 1.01–3.89, p = 0.044).

In multivariate analysis, only molecular subtypes and NLR^{low}/PLR^{low} retained their significance (Table 3). Compared to patients with luminal HER2-negative tumors, those with triple-negative or HER2-positive disease had a 3-fold increase in pCR (OR 2.99, 95% CI 1.31–6.84, p = 0.009). Similarly, compared to patients with a pro-inflammatory phenotype (NLR^{high} and/or PLR^{high}), those with a pro-immunogenic phenotype (NLR^{low}/PLR^{low}) showed more than 2-fold higher chance to achieve pCR (OR 2.29, 95% CI 1.22–4.27, p = 0.009).

Table 3
Association of patient/tumor characteristics to pCR in multivariate analysis.

Variable	OR	95% CI	p value*
TN/HER2+ *vs* Luminal HER2-	2.99	1.31–6.84	**0.009**
Grading G2/G3 *vs* G1	1.68	0.72–3.92	0.230
Ki67 ≥ 14% *vs* Ki67 < 14	1.30	0.64–2.67	0.459
NLR^{low}/PLR^{low} *vs* NLR^{high} and/or PLR^{high}	2.29	1.22–4.27	**0.009**
Chemio + Trastuzumab *vs* Chemio only	0.77	0.40–1.51	0.460

* Significant values are indicated in bold.

4. Discussion

In this study we provide evidence that low levels of peripheral NLR and PLR, evaluated at the initiation of NACT in patients with breast cancer, are predictive of pCR. We retrospectively calculated NLR and PLR from the blood cell count of 373 patients who were going to start NACT and fixed cut-off values according to ROC curve analysis. We found that patients who contextually presented NLR $\leq$2.42 and PLR $\leq$104.72, i.e. with both parameters low, had a significantly higher rate of pCR compared to patients with at least one of these parameters high.

In the last years several studies have revealed a fundamental contribution of the immune system to tumor response to chemotherapy. It has been demonstrated that some chemotherapeutical agents and oncolytic virus are able to elicit the release of antigens and pro-immunogenic factors that boost immune activation and, as a consequence, amplify anti-cancer response by triggering immunogenic cell death (ICD) [41–44].

Although breast cancer has been considered for several years an immune elusive tumor [15], a role of the immune system has recently emerged also in this neoplasm. Innate and adaptive immunity, as well as TILs, have been found to determine not only a better response to monoclonal antibodies, such as Trastuzumab and Pertuzumab [20,45,46], but even to chemotherapy [19,47,48] and radiotherapy [49]. Moreover, the presence of TILs has been correlated with a lower probability of recurrence and longer survival, regardless of breast cancer subtype [50].

However, the role of peripheral systemic immunity on cancer response to chemotherapy appears less clear. In different malignancies peripheral indicators of immunity and inflammation balance, such as NLR and PLR, correlate with survival and response to therapies [51–55]. In a recent paper by Vernieri and colleagues, NLR and PLR were found to correlate with response to platinum compounds in metastatic triple negative breast cancer [56]. Different studies have also explored immunity/inflammation indicators in breast cancer response to NACT [24–33]. Despite the number of studies published thus far, no clear indication of a role for peripheral systemic immunity has emerged, due in part to the lack of standardized cut-off values and the limited number of patients enrolled.

Our study investigated the predictive value of NLR and PLR for pCR in 373 patients affected by breast cancer and candidates to NACT. All the blood cell counts were performed centrally at our institutional laboratory. At the best of our knowledge this is the largest study for number of patients enrolled in the neoadjuvant setting of breast cancer. Several findings have emerged from our work.

First, NLR and PLR were not affected by clinical/pathological characteristics of patients, with the exception of age, i.e. older women (>50 years) had significantly higher frequencies of NLR^{low}, and stage, i.e. cT3/cT4 tumors showed a higher proportion of PLR^{high}.

Second, NLR and PLR were not significantly associated with pCR when analyzed separately, although higher rates of pCR were observed for both NLR^{low} and PLR^{low} (OR 1.53, $p = 0.125$, and OR 1.59, $p = 0.084$, respectively).

Third, when combining NLR and PLR, patients with NLR^{low}/PLR^{low} achieved a significantly higher rate of pCR compared to those with NLR^{high}/PLR^{high} (OR 1.94), indicating that an immunogenic phenotype, but not an inflammatory one, is involved in chemotherapy response. Since inflammation requires a huge recall of neutrophils, platelets and others cells from bone marrow [57,58], the combination of different biomarkers could better define the peripheral immune phenotype of patients. Interestingly, a recent paper published by Li and colleagues showed that the systemic immune-inflammation index, calculated taking into account the pre-therapeutic peripheral blood platelet, neutrophil, and lymphocyte counts, could discriminate a population of newly diagnosed elderly cancer patients with a poor prognosis [59].

Finally, the predictive value of NLR^{low}/PLR^{low} for pCR was independent from common prognostic factors such as grading, Ki67, and molecular subtype. This latter, particularly referred to HER2-positive and triple-negative tumors, is a well-known factor associated with pCR and, even in our study, resulted an independent variable able to predict pCR.

An important biological conundrum arise from the results of this study: does NLR^{low}/PLR^{low} in the peripheral blood reflect the antitumor immunity status of the patients? To clarify this issue it would be decisive to correlate NLR and PLR with the presence of TILs in the tumor microenvironment. Unfortunately these data were not available in our database and would require *ad hoc* investigation.

Adequately sized, prospective clinical trials are needed to understand if NLR/PLR could become a factor to take into account, along with cancer molecular subtype, to refer patients with breast cancer to NACT. Additional studies could also shed light on a possible role of the addition of anti-inflammatory drugs to standard chemotherapy in order to switch NLR/PLR from an inflammatory to an immunogenic phenotype, and thus potentially increasing the rate of pCR.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The work has been approved by the local ethical committee.

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