“The neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios predict efficacy of platinum-based chemotherapy in patients with metastatic triple negative breast cancer”

1. Introduction

Breast cancer is the most common malignancy among women and also one of the leading causes of cancer related death (1).

Breast cancer is a heterogeneous disease, comprising multiple entities associated with distinctive histological and biological features, clinical presentations and behaviors and responses to therapy (2).

Among all breast cancers, 5 different molecular subtypes can be classified, basing on expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2): Luminal A, Luminal B, Her-2/neu, Basal-like- triple negative; Unclassified- normal breast-like (3,4).

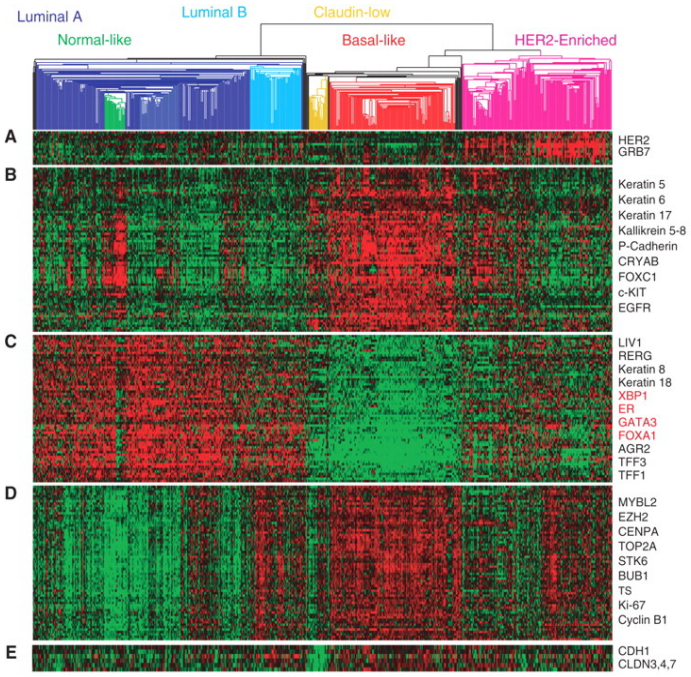

Figure 1. Breast cancer intrinsic subtypes by gene expression profile

It is generally recognized that currently, not all basal-like breast cancers display a triple negative phenotype and not all basal-like breast cancers are stratified as basal-like tumors by gene expression profiling (5).

Triple negative breast cancer is the term used to describe breast cancers that lack estrogen- and progesterone-receptor expression and do not overexpress HER2 by immunohistochemistry.

The definition of negative ER/PR status is not concordant in the literature, with some definitions considering ER/PR expression to be significant only if at least 10% of tumor cells express the receptors. However, the St. Gallen guidelines (6), the American

Society of Clinical Oncology (7), and the American College of Pathology (8) have defined triple negative breast cancer as breast cancer with less than 1% of tumor cells expressing ER and PR via immunohistochemistry.

It is widely accepted that TNBC is a very inhomogeneous group; using gene expression analyses, 6 distinct TNBC subtypes have been recently identified, each displaying a unique biology: basal-like 1 and 2 (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR) subtype, the last being characterized by androgen receptor (AR) signaling (9).

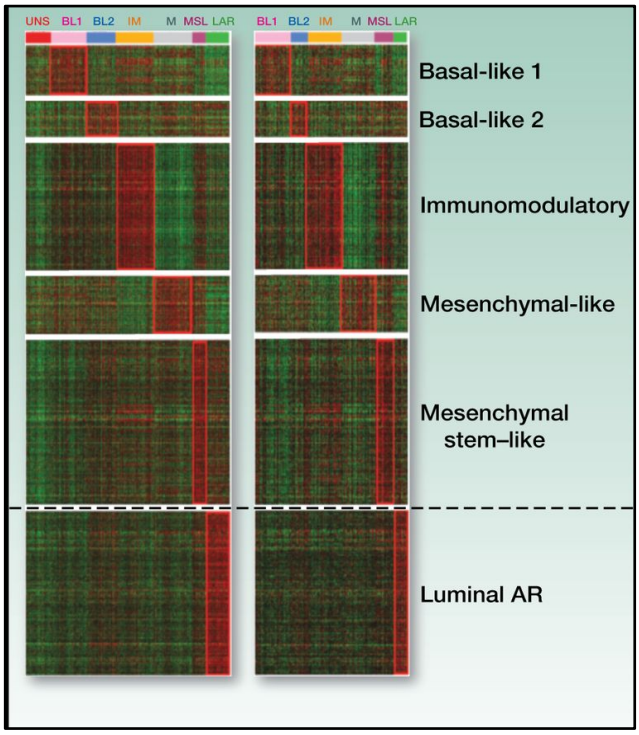

Figure 2. Gene expression subtype classifications of triple negative breast cancer (TNBC).

Metastatic triple negative breast cancer accounts for 10–20% of all metastatic breast cancer cases, and is characterized by an aggressive course, the lack of therapeutic targets and high lethality (10,11).

Even in recently published prospective trials, median progression free survival (PFS) and overall survival (OS) did not exceed 6 months and 12–18 months, respectively (12,13).

Cytotoxic chemotherapy still remains the mainstay of treatment for triple negative breast cancer, both in adjuvant and in metastatic setting, and particularly taxane (paclitaxel, docetaxel, nabpaclitaxel) and

platinum salts (cisplatin and carboplatin) are considered among the most effective compounds in this breast cancer subgroup (12-17).

Especially, platinum salts (specifically carboplatin) have been compared to docetaxel in metastatic triple negative breast cancer patients bearing germline mutations in *BRCA1* or *BRCA2* genes, showing greater response and progression free survival in favor of carboplatin over docetaxel, demonstrating clinical utility for treatment selection in this setting (14).

These results suggest that platinum compounds could be preferred treatment options in this patient population (14).

While such results await confirmation in larger studies, new predictive biomarkers are needed that are low-cost, routinely assessable with standardized techniques, well reproducible across different laboratories, and capable of predicting benefit from platinum-based chemotherapy.

Many evidences highlight the importance of immune system activation in control of this particular breast cancer subgroup: first, tumor-infiltrating lymphocytes (TILs) and other immune markers correlate with higher rates of pathological complete response (pCR), as well

as with better patient disease-free survival (DFS) and overall survival (OS), after neo-adjuvant chemotherapy (18-21); second, the programmed death 1 (PD-1) inhibitor Pembrolizumab (Keytruda®, Merck) and the PD-1 ligand (PD-L1) inhibitor Atezolizumab (Tecentriq®, Roche) are active in subgroups of heavily pre-treated TNBC patients (22-27). Moreover, in a recent phase 3 clinical trial, the combination of nab-paclitaxel with atezolizumab as first-line treatment in triple negative metastatic breast cancer patients led to significantly longer progression-free survival than was seen with placebo plus nab-paclitaxel in both the intention-to-treat population and the subgroup of patients with PD-L1–positive tumors (27); to date, chemotherapy plus atezolizumab combinations are being evaluated in larger, prospective, phase 3 clinical trials [NCT03125902, NCT03371017, http://clinicaltrials.gov].

Recent studies have revealed the prognostic role of parameters that reflect systemic inflammation or the status of antitumor immunity, such as the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), in different solid malignancies, including colorectal, renal and lung carcinomas (28, 29).

High NLR has also been associated with an increased risk of death in heterogeneous breast cancer patient populations (30), including patients with limited-stage triple negative breast cancer (31, 32).

However, none of these studies specifically assessed the role of NLR in metastatic triple negative breast cancer and, even in those studies including different proportions of patients with metastatic breast cancer (4.2–13.9%), subgroup analyses in specific tumor biology subtypes, or based on the type of chemotherapy, were not reported.

In this work, we assessed for the first time the potential role of NLR and PLR as biomarkers predictive of PFS in metastatic triple negative breast cancer patients treated with platinum-based chemotherapy. As a control population, we analyzed data from patients with hormone receptor positive, HER2 negative (ER positive/HER2 negative) metastatic breast cancer treated with the same platinum-containing regimens. To discriminate between a predictive and a purely prognostic role of these parameters, we also evaluated patient overall survival.

2. Methods

2.1 Study setting

This was a monocentric, retrospective study on patients with metastatic triple negative breast cancer treated between July 2007 and July 2017 at Fondazione IRCCS Istituto Nazionale dei Tumori placed in Milan, Italy, with platinum-based chemotherapy. The study was performed in accordance to relevant guidelines and regulations. Patients alive at the time of data collection and/or analysis signed an informed consent for the use of their personal data for research purposes.

Eligibility criteria were: (a) age ≥18 years; (b) pathologically or cytologically confirmed diagnosis of unresectable, locally recurrent or metastatic TNBC, as defined by ER <1% and PgR <1% expression at immunohistochemistry (IHC) analysis and an IHC score for HER2 of 0, 1+, or 2+ with negative in situ hybridization (ISH); (c) ECOG performance status (PS) of 0–1; (d) treatment with one the following chemotherapy schedules: carboplatin area under the concentration-time curve (AUC) of 2 plus paclitaxel 80 mg/m2, or carboplatin AUC 2 plus gemcitabine 800 mg/m2, both given on days 1 and 8 of every-three weeks cycles; (e) availability of baseline (pre-

treatment) absolute peripheral blood neutrophil, lymphocyte and platelet counts; (f) available information about previous treatment(s) for limited-stage or advanced disease; (g) available information on the date of disease progression and on patient status (alive versus dead; date of death); (h) absence of acute infections or documented bone marrow infiltration at the time of peripheral blood cell count assessment. As a control population, we selected patients with ER positive/HER2 negative metastatic breast cancer treated with the same platinum-containing regimens in the July 2007-July 2017 decade at Fondazione IRCCS Istituto Nazionale dei Tumori, Milan (Italy).

All subjects fulfilling these criteria were evaluated, regardless of line of treatment for metastatic breast cancer.

2.2 Objectives.

The main objective of the study was to investigate the potential association between baseline NLR/PLR and clinical outcome. The primary clinical endpoint was progression free survival (PFS), as defined as the time between treatment initiation and disease progression or death from any cause. Overall survival (OS) was a

secondary endpoint, and was defined as the time between treatment initiation and death from any cause.

2.3 Assessment of response.

Response was assessed every three cycles of chemotherapy, whereas tumor re-evaluation was anticipated in patients with worsening symptoms or other signs suggestive of progressive disease (PD). Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST version 1.1). Patients with only superficial, measurable disease were evaluated also clinically by measuring lesion diameters (with calipers) every three weeks.

2.4 Evaluation of biomarkers.

We collected data on absolute counts of peripheral blood neutrophils, lymphocytes, platelets and monocytes. We calculated the following parameters:

(a) NLR by dividing neutrophil by lymphocyte counts;
(b) PLR by dividing platelet by lymphocyte counts.

As an exploratory analysis, we also assessed the lymphocyte-to-monocyte ratio (LMR).

Blood parameters were evaluated before initiation of platinum-based chemotherapy. Parameters measured

within one month before the initiation of platinum-based chemotherapy were considered acceptable, provided that the patient was not receiving any concomitant anticancer treatment. Patients whose blood parameters were measured more than one month before the date of chemotherapy initiation, or after having received the first dose of platinum-based treatment, were excluded from this study. As an exploratory analysis, we also evaluated the NLR and PLR before the administration of the third treatment cycle.

2.5 Genetic analysis.

Analysis of *BRCA1/2* germline mutations was carried out on the DNA extracted from peripheral blood leukocytes. All coding exons and flanking regions of *BRCA1* and *BRCA2* genes were sequenced through direct sequencing, followed in most cases by multiple ligation-dependent probe amplification (MLPA) to detect large genomic rearrangements. Identified genetic variants were classified according to the IARC 5-tier scheme, following the guidelines of the Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA; http://enigmaconsortium.org/) (33).

2.6 Statistical analysis.

Patients' characteristics were analyzed by descriptive statistics. The $\chi 2$ or Fisher's exact tests were used to assess the association between categorical variables, while linear correlation was used for continuous variables. Progression free survival (PFS) and overall survival (OS) were calculated according to the Kaplan-Meier method, and the log-rank test was used to compare survival between different patient populations.

The impact of known prognostic factors on PFS was first assessed at univariate analysis. Covariates significantly associated with the risk of progression ($p < 0.1$) were then included in a Cox proportional hazard model to assess their independent association with survival.

Based on previously published data, the following categorical covariates were tested: (a) previous exposure to taxanes (yes vs no); (b) visceral disease (yes vs no); (c) the number of metastatic sites (1–2 vs >2); (d) having received maintenance treatment (yes vs no). The type of chemotherapeutical agent combined with carboplatin (i.e. gemcitabine vs paclitaxel) was also tested as a covariate. All statistical analyses were

performed using the software R (version 3.3.2 (2016-10-31)), while the package “survival” was used for survival analyses. A p value of 0.05 was chosen as a threshold level for statistical significance.

3. Results

3.1 Patient characteristics.

Between July 2007 and July 2017, 62 metastatic triple negative breast cancer patients were treated at Fondazione IRCCS Istituto Nazionale dei Tumori, Milan (Italy) with platinum-based chemotherapy combinations. Among these patients, 57 fulfilled the inclusion criteria and were evaluable for the biomarkers of interest. At the moment of data lock and analysis, 53 (93%) patients had progressed and 48 (84%) had died.

Characteristics of evaluated patients are described in Table 1.

	TNBC	ER/PgR-positive HER2-negative
N. pts	57	148
Median age (years, range)	56 (33.7–78.9)	58 (29.8–79.3)
Previous taxane exposure		
Yes	43 (75.4%)	102 (68.9%)
No	14 (24.6%)	46 (31.1%)
N. chemotherapy line		
1^{st}–2^{nd}	57 (100%)	104 (70.3%)
$>2^{nd}$	0 (0%)	44 (29.7%)
N. disease sites:		
1–2 sites	36 (63.2%)	88 (59.5%)
>2 sites	21 (36.8%)	60 (40.5%)
Presence of visceral disease	37 (65%)	99 (66.9%)
Type of treatment		
paclitaxel	48 (84.2%)	112 (75.7%)
gemcitabine	9 (15.8%)	36 (24.3%)
Maintenance therapy	14 (24.6%)	65 (43.9%)

Table 1. Characteristics of patients with metastatic TNBC and the ER-PgR positive/HER2 negative BC control population.

Median age among metastatic triple negative breast cancer (TNBC) patients was 56 years (range 33.7–78.9).

Most patients (84%) received chemotherapy with carboplatin plus paclitaxel, while the remaining ones (16%) were treated with the carboplatin plus gemcitabine combination. All metastatic TNBC patients received platinum-containing chemotherapy as their first- (88%) or second-line (12%) treatment.

In the control population of patients with ER positive/HER2 negative metastatic breast cancer, median age was 58 years (range 29.8–79.3); most patients (75.7%) were treated with carboplatin plus paclitaxel and the remaining ones (24.3%) received carboplatin plus gemcitabine. Of these patients, 70.3% received platinum-based chemotherapy as their first- or second-line therapy.

3.2 Impact of NLR and PLR on PFS

Median progression free survival (PFS) in the triple negative breast cancer population was 204 days.

Higher neutrophils (4000/μl) were associated with non-significantly lower PFS ($p = 0.081$), while platelets above 300000/μl or lymphocytes below 1500/μl

correlated with significantly shorter PFS (p = 0.041 and p = 0.005, respectively) (Fig. 3)

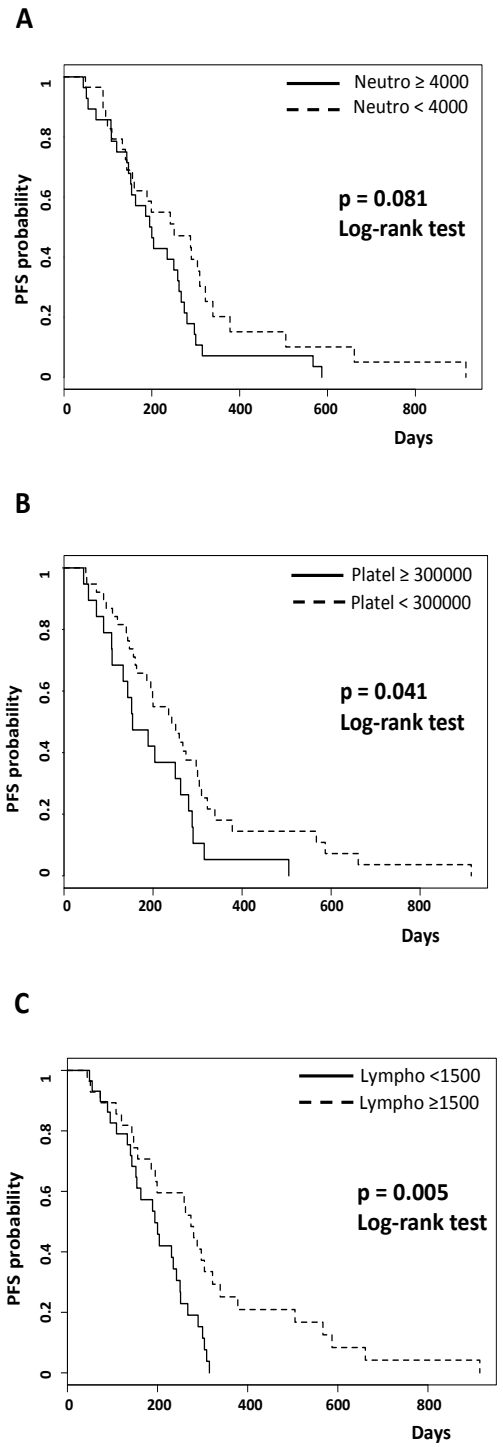

Figure 3. Kaplan-Meier curves of progression free survival (PFS) according to baseline neutrophils (A), platelets (B) and lymphocytes (C) counts

We then investigated the potential predictive role of NLR and PLR. Based on previous studies, we chose a NLR threshold of 2.5 and a PLR threshold of 200 (30, 34). Both NLR and PLR were inversely associated with patient age ($p < 0.001$ and $p = 0.048$, respectively), while they did not correlate with previous taxane exposure ($p = 0.076$ and $p = 0.68$, respectively), presence of visceral disease ($p = 0.9$ and $p = 0.6$, respectively), type of platinum-based chemotherapy received ($p = 0.48$ and $p = 0.7$, respectively) and number of metastatic sites ($p = 0.22$ and $p = 0.39$, respectively) (shown in Table 2).

	Total (n = 57)	**NLR <2.5 (n = 25)**	**NLR ≥2.5 (n = 32)**		**PLR <200 (n = 34)**	**PLR ≥200 (n = 23)**	
	N (%)	**N (%)**	**N (%)**	**p value**	**N (%)**	**N (%)**	**p value**
Pts age							
<50 yrs	21 (36.8%)	3 (5.2%)	18 (31.6%)	**<0.001**	9 (15.8%)	12 (21%)	**0.048**
>50 yrs	36 (63.2%)	22 (38.6%)	14 (24.6%)		25 (43.9%)	11 (19.3%)	
Previous taxane							
Yes	43 (75.4%)	16 (28%)	27 (47.4%)	0.076	25 (43.8%)	18 (31.6%)	0.68
No	14 (24.6%)	9 (15.8%)	5 (8.8%)		9 (15.8%)	5 (8.8%)	
Visceral disease							
Yes	37 (64.9%)	16 (28%)	21 (36.8%)	0.9	23 (40.3%)	14 (24.6%)	0.6
No	20 (35.1%)	9 (15.8%)	11 (19.4%)		11 (19.3%)	9 (15.8%)	
N. metastatic sites							
1–2	36 (63.2%)	18 (31.6%)	18 (31.6%)	0.22	23 (40.3%)	13 (22.8%)	0.39
>2	21 (36.8%)	7 (12.3%)	14 (24.5%)		11 (19.3%)	10 (17.6%)	
ChT type							
Taxane	48 (84.2%)	20 (35.1%)	28 (49.1%)	0.48*	28 (49.1%)	20 (35.1%)	0.7*
Gemcitabine	9 (15.8%)	5 (8.8%)	4 (7%)		6 (10.5%)	3 (5.3%)	
Maintenance ChT							
Yes	14 (24.6%)	10 (17.6%)	4 (7%)	**0.017**	11 (19.3%)	3 (5.3%)	0.097
No	43 (75.4%)	15 (26.3%)	28 (49.1%)		23 (40.3%)	20 (35.1%)	

Table 2. Characteristics of metastatic TNBC patients by NLR and PLR.

Pts = patients; ChT = chemotherapy. *Fisher's exact test.

Finally, high NLR correlated with lower probability of receiving maintenance chemotherapy ($p = 0.017$), while PLR did not ($p = 0.097$).

Median PFS was 304 days in patients with NLR < 2.5 and 158 days in those with NLR ≥ 2.5 (HR 3.25, 95% CI 1.72–6.25; $p < 0.001$) (Fig. 4A). Similar results were obtained by choosing a threshold of 3.3 (274 vs 148 days, $p < 0.0001$) or 2 (309 vs 186 days, $p = 0.0015$) (data not shown). Regarding the PLR, PFS was longer in patients with baseline PLR < 200 as compared to PLR ≥ 200 (HR 2.75, 95% CI 1.52–4.99; $p < 0.001$) (Fig. 4B). Baseline LMR ≥ 4.5 was also associated with significantly better PFS in TNBC patients, although the association was less strong than in the case of NLR and PLR (HR 2.22, 95% CI 1.22–4.05, $p = 0.009$; data not shown).

When the same parameters were evaluated before the administration of the third treatment cycle, NLR < 2.5 was still associated with reduced risk of disease progression (140 vs 262 days, $p < 0.001$), while PLR < 200 was not (259 vs 204 days, $p = 0.48$) (data not shown).

In the control population of ER positive/ HER2 negative metastatic BC patients, median PFS was 220

days. Baseline higher NLR and PLR were non-significantly associated with the risk of disease progression (p = 0.21 and p = 0.29) (Fig. 4 C and D).

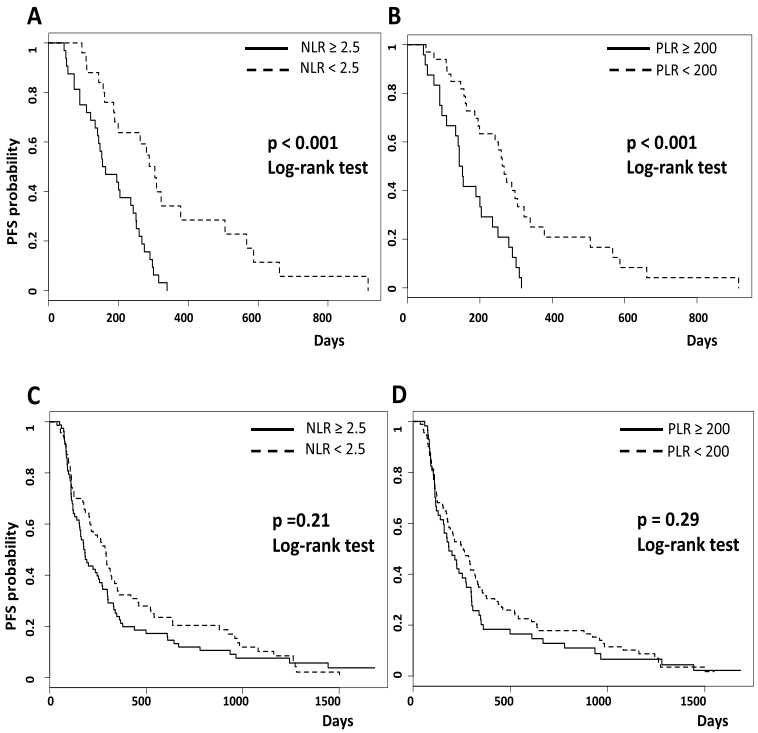

Figure 4. Kaplan-Meier curves of progression free survival (PFS) of TNBC (A and B) and ER positive/HER2 negative (C and D) mBC patients according to baseline NLR (A and C) and PLR (B and D).

3.3 Independent predictive role of NLR and PLR on progression free survival (PFS).

Factors associated with the risk of disease progression in metastatic TNBC were: previous exposure to taxanes, the presence of visceral metastases, having received maintenance chemotherapy, NLR ≥ 2.5 and PLR ≥ 200 (Fig. 5A). As previously described27, NLR and PLR positively correlated with each other (Pearson

coefficient regression = 0.49; $p < 0.001$); therefore, only one of these parameters was evaluated at multivariable analysis.

In the multivariable model including NLR as a covariate, NLR ≥ 2.5 was associated with significantly lower PFS (HR 2.65; $p = 0.004$), while other covariates were not statistically significantly associated with PFS (Fig. 5B).

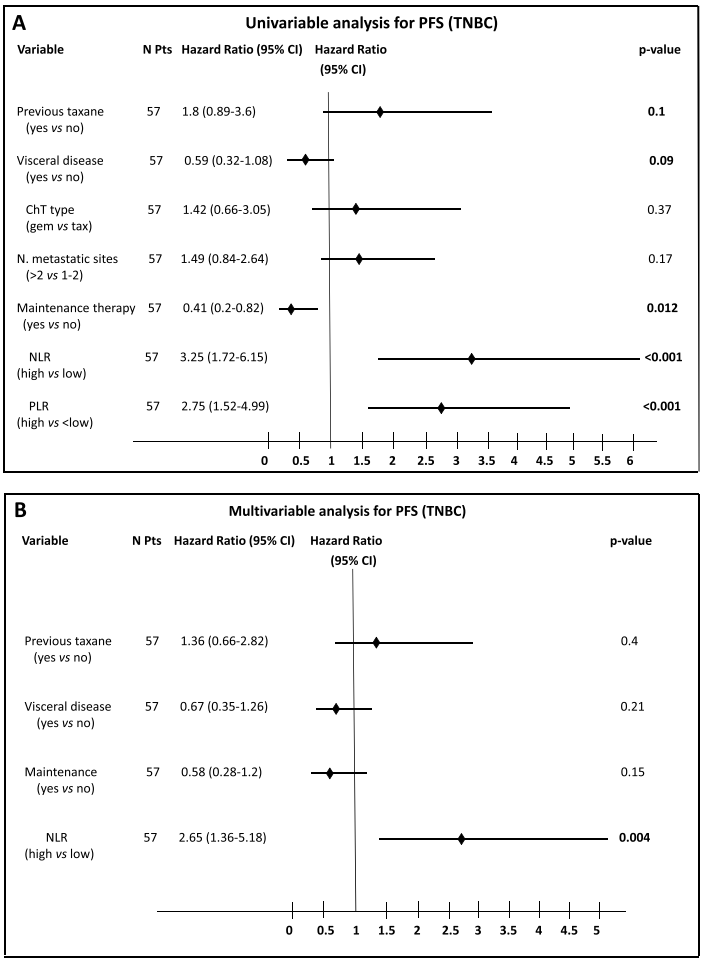

Figure 5. Forest plot illustrating the results of univariable (A) and multivariable (B) analysis of covariates associated with the risk of disease progression in mTNBC.

When we tested PLR at multivariable analysis, PLR ≥ 200 was independently associated with poorer patient prognosis

(HR 2.33; p = 0.007), while the other covariates were not, with the exception of maintenance chemotherapy, which correlated with reduced risk of progression (HR 0.45; p = 0.027) (data not shown).

Impact of peripheral blood parameters on overall survival (OS)

Median overall survival (OS) was 483 days in metastatic triple negative breast cancer patients and 653 days in the control population of ER positive/HER2 negative breast cancer patients.

In metastatic triple negative breast cancer, median overall survival (mOS) was significantly longer in patients with NLR < 2.5 compared to those with NLR ≥ 2.5 (p = 0.01), while PLR values were not associated with statistically significant differences in mOS (p = 0.14) (Fig. 6A and B).

In ER positive/HER2 negative breast cancer patients (control arm), both NLR < 2.5 and PLR < 200 were associated with significantly better mOS (p = 0.023 and p = 0.003, respectively) (Fig. 6 C and D).

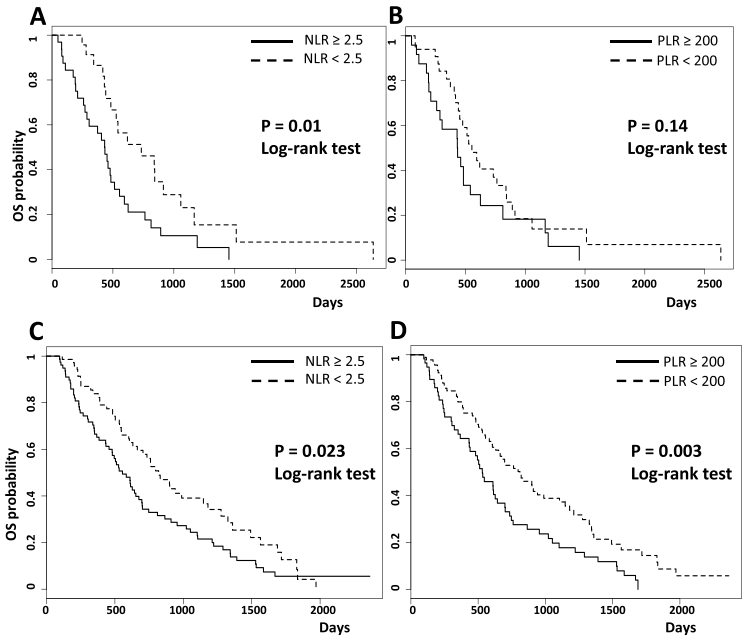

Figure 6. Kaplan-Meier curves of overall survival (OS) of TNBC (A and B) and ERpositive/HER2 negative (C and D) mBC patients according to baseline NLR (A and C) and PLR (B and D)

At univariate analysis, factors associated with worse overall survival in metastatic triple negative breast cancer patients were:

- NLR ≥ 2.5
- having received gemcitabine in combination with carboplatin,

while maintenance chemotherapy correlated with better progression free survival (Fig. 7A). At multivariable analysis, NLR ≥ 2.5 and gemcitabine treatment were independently associated with worse outcomes (Fig. 7B).

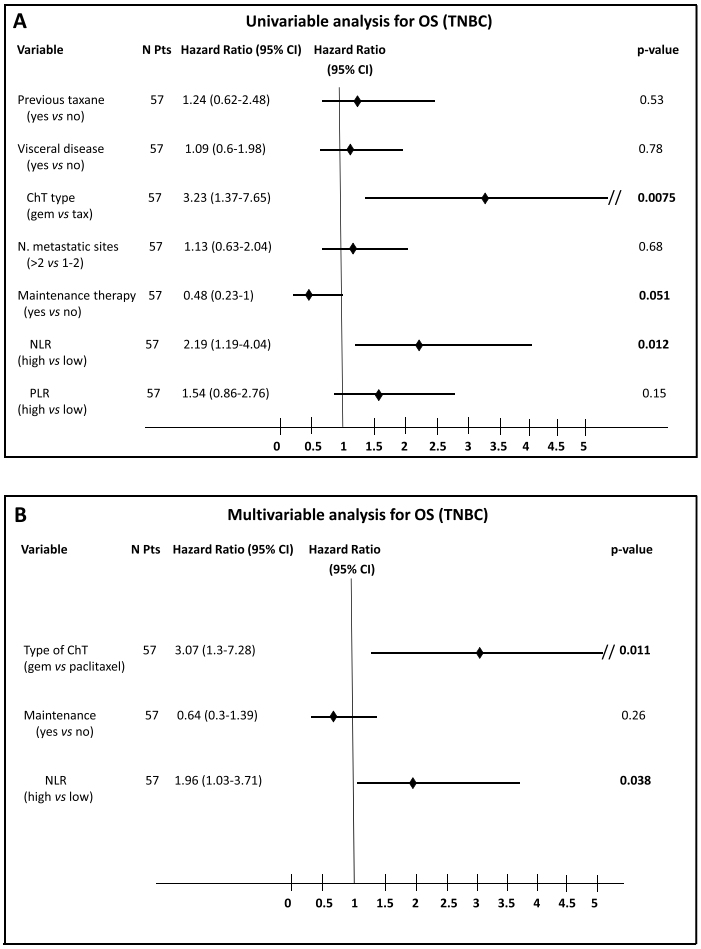

Figure 7: Forest plot illustrating the results of univariable (A) and multivariable (B) analysis of covariates associated with the overall survival in mTNBC.

In the control population, factors associated with worse overall survival were:

- NLR $\geq$ 2.5,
- presence of visceral disease,
- previous taxane exposure
- more advanced (>2nd) treatment lines,

while having received maintenance therapy correlated with lower risk of death (Fig 8A).

At multivariable analysis, high NLR and visceral involvement were independently associated with lower survival, while maintenance treatment correlated with better outcome (Fig. 8B).

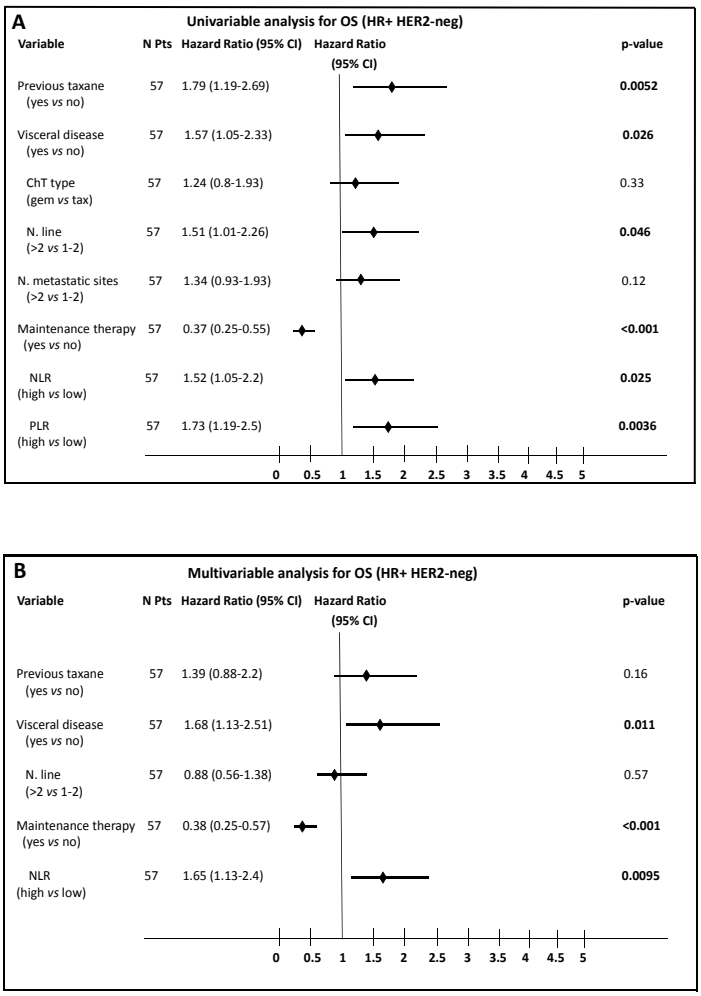

Figure 8. Forest plots illustrating the results of univariable (A) and multivariable (B) analysis of covariates associated with overall survival in ER positive/HER2 negative metastatic BC (control arm)

Impact of germline *BRCA 1/2* mutations on TNBC patient PFS

Among metastatic TNBC patients included in our study, only 19 had undergone analysis of germline

mutations of the *BRCA1/2* genes. Of them, 6 were carriers of a pathogenic germline *BRCA1/2* mutation, while the remaining 13 patients had wild-type *BRCA1/2* gene.

Median PFS was 126 days in carriers of *BRCA1/2* mutations and 163 days in non-mutated ones (HR = 1.09, 50% CI 0.38–3.12, p = 0.87).

4. Discussion

Although platinum-based chemotherapy is a standard treatment for metastatic triple negative breast cancer, many patients fail to respond, and no biomarkers are currently available to predict treatment effectiveness. In this study, we found for the first time an association between higher NLR or PLR and worse outcome in terms of progression free survival (PFS) in metastatic TNBC patients receiving carboplatin plus paclitaxel or carboplatin plus gemcitabine therapies, but not in a control population of ER positive/HER2 negative metastatic breast cancer patients treated with the same chemotherapy regimens.

The fact that specific blood cell populations reflect the inflammatory/immune contexture is quite a well-established concept. Indeed, high neutrophils can be associated with systemic inflammation or immune suppression (35); high platelets reflect systemic inflammation as well, but can be also associated with increased metastatization of neoplastic cells via platelet clots (30, 32); finally, low lymphocyte counts can be associated with impaired activation of adaptive immunity or poor nutritional status (33,34). In this study, the association between clinical outcomes and NLR/PLR was stronger than in the case of individual

cell counts. This is not surprising, since parameter combinations are more stable to changes in single parameters (e.g. neutrophils or platelets can increase during acute infections or glucocorticoid administration) and may capture more aspects of the tumor-immune system interplay. Notably, the HRs associated to NLR and PLR at multivariable analysis for PFS were similar, and these parameters also correlated with each other. This suggests that both NLR and PLR well reflect the inflammatory/ immune contexture in mTNBC, and may be redundant as predictive biomarkers.

In TNBC patients, higher NLR correlated with lower OS, but the association was weaker than in the case of PFS; moreover, no statistically significant association was found between PLR and OS. These results can be due to the fact that, different from PFS, OS is affected by the whole treatment course, including therapies administered after platinum-based chemotherapy; therefore, the association between NLR/PLR and PFS during platinum-based chemotherapy could be diluted by subsequent treatments for which the same parameters are not predictive.

Conversely, both NLR and PLR correlated with lower OS in the control population of ER positive/HER2

negative metastatic breast cancer patients, despite the fact that they were not predictive of PFS during platinum-based treatment. This could be due to the fact that NLR and PLR are associated to benefit from treatments administered after platinum containing chemotherapy; therefore, their effect may only emerge when evaluating OS as an endpoint. Alternatively, these parameters may be generally prognostic in ER positive/HER2 negative breast cancer, but not predictive of benefit from specific treatments. Based on our data, we are unable to discriminate between these two hypotheses. Studies conducted in recent years have revealed a previously unrecognized complexity of the number and functional status of different immune system subpopulations (36,37). For instance, circulating blood lymphocytes include phenotypically and functionally different cells, such as CD8+ cytotoxic lymphocytes, regulatory T cells and exhausted lymphocytes, whose balance can determine the predominantly antitumor or protumor activity of adaptive immunity35. In parallel, blood monocytes include both cells that differentiate into antitumor M1 macrophages at the tissue level, and myeloid-derived suppressive cells (MDSCs) that exert pro-tumor effects by inhibiting the activity of antitumor T lymphocytes

(37). Coherently with a previous research in localized TNBC (23), in this study we also found an association between the LMR and patient PFS, but this was weaker than in the case of NLR and PLR, and similar to the association between absolute lymphocyte counts and PFS. This finding suggests that monocytes may be poorly predictive of survival in metastatic TNBC.

Investigating the potential association between specific immune cell populations and patient prognosis could reveal more reliable and predictive parameters to improve treatment selection in metastatic TNBC. In this perspective, we recently started a prospective observational study to assess the role of baseline immunological parameters, as well as their on-treatment modifications, on the PFS of metastatic TNBC patients receiving platinum-based chemotherapy. In this study, we did not find any significant difference in PFS duration between patients with or without *BRCA1/2* germline mutations, which have recently emerged as associated with higher tumor response rates during carboplatin chemotherapy. However, the number of subjects amenable to this analysis was too low, and larger studies are required to assess the independent predictive role of NLR/PLR and BRCA1/2 mutations on the PFS of metastatic triple

negative breast cancer patients receiving platinum-based therapies.

Strengths of this study consist in the monocentric patient cohort, which guarantees more reproducible assessment of tumor response and coherent collection of patient laboratory data by different investigators, as well as the homogeneity of chemotherapy regimens used and the TNBC patient cohort. In particular, the fact that all TNBC patients received carboplatin-based chemotherapy as first- or second-line treatment makes our results more robust compared to previously published data in patients treated with different regimens in different treatment lines. Weaknesses of this study consist in the retrospective design, the limited number of TNBC patients included in the analysis and the lack of data on tumor-infiltrating immune cells, which did not allow us to establish a direct, mechanistic link between peripheral blood parameters and immune cell populations in tumor microenvironment or directly infiltrating the tumor. However, the NLR and PLR calculated at the initiation of platinum-based chemotherapy may reflect the systemic inflammatory and immunological status much more reliably than immune cells detected in tumor specimens biopsied/removed months/years before

treatment administration. Moreover, the NLR and PLR could offer a more global picture of the immune contexture, thus circumventing the spatial heterogeneity in tumor-infiltrating immune cell populations.

In conclusion, the NLR and PLR are predictive of benefit from platinum-containing chemotherapy specifically in metastatic triple negative breast cancer patients. They also confirm to have a generally prognostic role independently from tumor biology. If validated in larger prospective studies, these easy-to-measure parameters could be combined with emerging predictive biomarkers, such as germline or somatic *BRCA 1/2* gene mutations, to improve the selection of metastatic TNBC patients more likely to benefit from platinum-based chemotherapy.

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