Biomarkers Derived From Routine Blood Cell Counts Differentially Predict Disease-Free and Overall Survival After Neoadjuvant Treatment of Triple-Negative Breast Cancer

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Abstract

In recent years some serologic parameters emerged as potential prognostic factors. The neutrophil-to-lymphocyte ratio (NLR) has the most evidence; however, other serologic factors were also reported. The only established systemic treatment in triple-negative breast cancer (TNBC) is chemotherapy which is preoperatively applied more widely. For these patients few data are available on which serologic markers would be the best predictor for disease-free (DFS) and overall survival (OS). Data of 137 TNBC patients treated (2005-2016) with neoadjuvant chemotherapy at our center were analyzed. Beyond pathological factors, white blood cell (WBC), neutrophil (NE), lymphocyte (LY) and platelet (PL) counts, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII) were investigated. In univariate analysis, most parameters (NE1, LY1, NLR1, PLR1, SII1) measured at baseline and before the third cycle (NE3, LY3, etc.) of chemotherapy showed significant association with survival. After the exclusion of correlated variables, in multivariate analysis NLR1, Ki67 and pathological stage were independent predictors of DFS and OS. In an exploratory analysis new markers were found: dichotomization by NLR1xNLR3 and PL1/(PL3)^2 resulted in significantly different DFS of patients with low and high NLR1, respectively. A high PL3xLY3 level was an exclusive marker of relapse after pathological complete remission.

Introduction

The standardized death rate of breast carcinoma in Hungary is one of the highest among the European countries[1]. 15% of total cases are estrogen, progesterone and Her2 receptor negative (triple-negative, TNBC) tumors which have the most dismal prognosis. The only well-established medical treatment in these cases is chemotherapy, which is used at an increasing rate in the early stage of the disease. The neoadjuvant treatment may result in pathologic complete response (pCR), which is associated with excellent survival[2]. Various set ups, including dosage splitting of the given treatment before and after surgery[3], and immunotherapy may improve the outcome[4].

TNBC is a heterogeneous disease. Genetic analyses showed that TNBC can be divided in different subgroups (Lehman and Burstein classifications) showing different prognosis and chemo-sensitivity. Exploring differences may give the possibility to modify the treatment according to clinical data, to switch, to escalate or to de-escalate therapy. However, the treatment could not be directed solely on the basis of genetic features. In terms of survival there are several independent prognostic factors which may guide treatment planning, extent of the disease (TNM classification), differentiation and proliferation markers (grade and Ki67 labeling index), vascular invasion, pCR rate and the extent of tumor infiltrating lymphocytes (TILs)[5] are the most common predictors. In recent years lymphocyte (LY), neutrophil (NE), and platelet (PL) counts and their relative ratios emerged as potential prognostic and predictive factors probably characterizing the immune reaction of the host[6]. The neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR) may be independent prognostic factors in many tumor types[7,8]. There are also results suggesting that the pretreatment NLR value may predict the efficacy of chemotherapy in TNBC[9,10]. The NLR may have prognostic relevance in TNBC throughout the neoadjuvant therapy and even after completion of a curative treatment[11]. In a recent meta-analysis, the role of NLR in the neoadjuvant setting was inconclusive[12]. While most adjuvant analyses revealed independent association with survival, only half of the neoadjuvant studies involving all subtypes of breast cancer and one of two with TNBC[9,13] showed independent association with survival. Similarly, PLR was an independent predictive factor for survival in Patel's analysis[11], but not in Liu's data[14]. Choi et al reported that the changes in NLR may also correlat with survival[15]. The systemic immune-inflammation index (SII) (determined as PLxNE/LY), which can be associated with survival, was also investigated in several tumor types[16].

The aim of our investigation was to gather further information beyond pretreatment NLR on predictive and prognostic role of LY, NE, and PL measured before and during neoadjuvant chemotherapy of TNBC patients. Using these cell counts novel biomarkers were defined and tested.

Results

211 patients were eligible according to the selection criteria; however, the complete serologic data were available for 137 cases. The basic demographic, clinical and treatment characteristics with their prognostic role in survival are presented in Table 1 and 2.

Table 1

Clinicopathological and treatment characteristics before surgery and their effects on median disease-free (mDFS) and overall survival (mOS) of patients (n = 137).

Parameters	N (%)	mDFS (95% CI)	*p*	mOS (95% CI)	*p*
Age (yrs), median (range) 52 (25–78)		NR (33.1–82)		NR (71.6–99.5)	
< 52	66 (48)	78.9 (20.8–78.9)	0.546	NR (33.6–74.5)	0.427
≥ 52	71 (52)	NR (33.4–82)		NR (71.6–99.5)	
BMI, median (range) 26.3 (17.2–51.1), NA 2 (1%)					
< 26.3	68 (50)	38.1 (19.9–82)	0.14	NR (41-74.5)	0.34
≥ 26.3	67 (49)	NR (54.3–61.8)		NR (71.6–95.1)	
cT					
0/1/2/3	3 (2)/9 (7)/70 (51)/25 (18)	NR (56.1–82)	**0.001**	NR	**0.003**
4 *	30 (22)	13.8 (8-23.7)		30.2 (21.4–99.5)	
cN					
0/1/2	24 (18)/63 (46)/39 (28)	NR (44.9–82)	**0.009**	NR	**0.009**
3	11 (8)	19.4 (4.6–25.8)		15.9 (14.8–39.4)	
Clinical stage					
2a/2b/3a	13 (9)/48 (35)/41 (30)	NR (61.8–82)	**$5.6x10^{-4}$**	NR	**0.001**
3b/3c	24 (18)/11 (8)	16.5 (9.1–25.8)		30.2 (21.4–48.8)	
Type of neoadjuvant chemotherapy					
Anthracycline	71 (52)	NR (33.1–82)	0.672	NR (59-99.5)	0.693
Ant + tax/taxane	62 (45)/4 (3)	54.3 (25.8–54.3)		NR (42.9–74.5)	
The best response during neoadjuvant chemotherapy					
CR	25 (18)	NR	**10^{-5}**	NR	**$7x10^{-5}$**
PR	84 (61)	82 (27.8–82)		NR (50.9–74.5)	
SD	15 (11)	56.1 (10.5–56.1)		95.1 (20.9–95.1)	
PD	13 (9)	8 (4.6–13.8)		24.6 (14.7–27.8)	
Cyles of neoadjuvant + adjuvant chemotherapy					
< 6	21 (15)	10.1 (4.8–82)	**0.002**	16.9 (14.7–36.5)	**0.005**
≥ 6	116 (85)	NR (44.9–78.9)		NR	

* 16 (12%) patients with 4d.

Ant, anthracycline; B-conserving, breast-conserving; BMI, body mass index; tax, taxane; CI, confidence interval; CR, complete response; NA, not available; NR, not reached; PD, progressive disease; PR, partial response; SD, stable disease.

Table 2

Clinicopathological and treatment characteristics during and after surgery and their effects on median disease-free (mDFS) and median overall survival (mOS) of patients (n = 137).

Parameters	N (%)	mDFS (95% CI)	*p*	mOS (95% CI)	*p*
Type of surgery					
Mastectomy	104 (76)	56.1 (24.5–61.8)	**10^{-10}**	NR (50.9–99.5)	**0.029**
B-conserving	28 (20)	NR (82–82)		NR	
None	5 (4)	4.2 (1-9.8)		9 (5.9–25.8)	
Free margin (mm), median (range) 5 (0–25), NA 52 (38%)					
< 2.1	27 (20)	16.2 (7.6–19.9)	**$3.8x10^{-5}$**	27.8 (20.9–36.5)	**10^{-4}**
≥ 2.1	58 (42)	NR (61.8–61.8)		NR	
Grade					
2	9 (7)	NR	0.446	99.5 (99.5–99.5)	0.511
3	128 (93)	82 (30.4–82)		NR (59-95.1)	
MAI, median (range) 24 (0-106), NA 30 (22%)					
< 33	68 (50)	NR (30.4–82)	**0.031**	NR (42.9–99.5)	0.058
≥ 33	39 (28)	23.4 (13.8–56.1)		48.8 (23.5–95.1)	
Necrosis, NA 3 (2%)					
No	57 (42)	NR	**0.001**	NR	**0.001**
Yes	77 (56)	27.8 (17-61.8)		52.9 (35.5–99.5)	
Vascular invasion, NA 2 (1%)					
No	88 (64)	NR	**$1.2x10^{-5}$**	NR	**$2x10^{-6}$**
Yes	47 (34)	17 (12-25.8)		33.4 (23.1–41)	
Ki67 (%), median (range) 70 (3-100), NA 38 (28%)					
< 50	24 (18)	NR	**0.005**	NR	**0.013**
≥ 50	75 (55)	82 (23.8–82)		NR (36.8–95.1)	

Ant, anthracycline; B-conserving, breast-conserving; CI, confidence interval; MAI, mitotic activity index; NA, not available; NR, not reached.

Parameters	N (%)	mDFS (95% CI)	*p*	mOS (95% CI)	*p*
pT, NA 5 (4%)					
0/1mi/1a/1b/1c	43 (31)/5 (4)/1 (1)/5 (4)/23 (17)	NR	**$3.4x10^{-5}$**	NR	**$3x10^{-5}$**
2/3/4a/4d	33 (24)/16 (12)/5 (4)/1 (1)	20.8 (13.8–33.1)		33.6 (27-99.5)	
pN, NA 5 (4%)					
0	72 (53)	NR	**$8.2x10^{-5}$**	NR	**$4x10^{-5}$**
1/2/3	35 (26)/12 (9)/13 (9)	23.8 (15.6–33.4)		40.5 (31.2–95.1)	
Pathological stage					
0/1a	38 (28)/13 (9)	NR	**$7x10^{-7}$**	NR	**$2x10^{-7}$**
1b/2a/2b/3a/3b/3c/4	2 (1)/29 (21)/14 (10)/16 (12)/4 (3)/13 (9)/8 (6)	23.4 (13.8–33.1)		39.4 (30.3–71.6)	
Type of adjuvant chemotherapy					
Ant/taxane	32 (23)/ 30 (22)	NR (61.8–82)	**$1.5x10^{-4}$**	NR	**0.003**
Ant + taxane	6 (4)	19.4 (9.1–26.3)		17.1 (16.9–23.1)	
None	69 (50)				
Adjuvant radiotherapy					
No	57 (42)	NR (23.7–82)	0.708	99.5 (41-99.5)	0.465
Yes	80 (58)	78.9 (30-78.9)		NR (71.6–95.1)	

Ant, anthracycline; B-conserving, breast-conserving; CI, confidence interval; MAI, mitotic activity index; NA, not available; NR, not reached.

The investigated markers and their association with DFS and OS are shown in Table 3 and 4. At the time of surgery 8 patients were in stage IV due to progression, 75% of them had the NLR1 level ≥ 2.76.

Table 3
Blood cell counts and derived markers at the start of the 1st cycle and their association with median disease-free (mDFS) and overall survival (mOS).

Parameters	N (%)	median (range)	mDFS (95% CI)	*p*	mOS (95% CI)	*p*
WBC count (WBC1)		8 (3.45–14.09)				
< 8.32	74 (54)		NR (55.7–82)	**0.027**	NR	0.138
≥ 8.32	63 (46)		44.9 (23.7–78.9)		74.5 (35.5–99.5)	
Platelet count (PL1)		275 (96–862)				
< 294	83 (61)		61.8 (27.8–82)	0.398	95.1 (42.9–95.1)	0.238
≥ 294	54 (39)		NR (23.8–30)		NR	
Neutrophil count (NE1)		5.26 (1.73–10.8)				
< 5.97	83 (61)		NR (82–82)	**0.003**	NR	**0.035**
≥ 5.97	54 (39)		23.8 (14.6–33.4)		42.9 (31.4–99.5)	
Lymphocyte count (LY1)		1.89 (0.78–4.24)				
< 2.34	99 (72)		37.5 (20.8–82)	**0.005**	95.1 (39.4–99.5)	**0.005**
≥ 2.34	38 (28)		NR		NR	
NE1/LY1 (NLR1)		2.54 (0.85–8.72)				
< 2.76	78 (57)		NR	**$4x10^{-5}$**	NR	**$4.6x10^{-4}$**
≥ 2.76	59 (43)		23.7 (12.2–38.1)		41 (28.9–95.1)	
PL1/LY1 (PLR1)		142 (51.6–391)				
< 118.4	44 (32)		NR	**0.007**	NR	**0.022**
≥ 118.4	93 (68)		37.5 (20.8–82)		95.1 (39.4–99.5)	
PL1xNE1/LY1 (SII1)		704 (130–3951)				
< 613	53 (39)		NR	**0.001**	NR	**0.005**
≥ 613	84 (61)		26.3 (16.5–78.9)		74.5 (31.4–99.5)	

CI, confidence interval; NR, not reached.

Table 4
Blood cell counts and derived markers at the start of the 3rd cycle and their association with median disease-free (mDFS) and overall survival (mOS).

Parameters	N (%)	median (range)	mDFS (95% CI)	*p*	mOS (95% CI)	*p*
WBC count (WBC3)		6.31 (2.3-15.17)				
< 6.78	77 (56)		NR (37.5–82)	0.285	NR	0.109
≥ 6.78	60 (44)		44.9 (23.7–61.8)		74.5 (36.5–95.1)	
Platelet count (PL3)		329 (151–737)				
< 382	100 (73)		54.3 (23.7–78.9)	0.106	99.5 (47.9–99.5)	0.124
≥ 382	37 (27)		NR (61.8–82)		NR	
Neutrophil count (NE3)		3.76 (0.99–14.2)				
< 4.43	76 (55)		NR (78.9–82)	**0.04**	NR	**0.012**
≥ 4.43	61 (45)		33.4 (19.9–61.8)		52.9 (33.4–95.1)	
Lymphocyte count (LY3)		1.55 (0.34–3.57)				
< 1.55	68 (50)		30.4 (22.8–82)	**0.021**	74.5 (36.8–95.1)	0.052
≥ 1.55	69 (50)		NR (78.9–78.9)		NR	
NE3/LY3 (NLR3)		2.56 (0.57–21.9)				
< 2.34	61 (45)		NR	**$4x10^{-4}$**	NR	**$2.7x10^{-4}$**
≥ 2.34	76 (55)		26.3 (16.6–56.1)		50.9 (30.6–95.1)	
PL3/LY3 (PLR3)		218 (60.1–1638)				
< 225	73 (53)		NR (61.8–78.9)	**0.028**	NR	0.118
≥ 225	64 (47)		27.8 (16.6–82)		99.5 (31.2–99.5)	
PL3xNE3/LY3 (SII3)		854 (152-12330)				
< 846	66 (48)		NR	**0.001**	NR	**0.003**
≥ 846	71 (52)		30 (19.4–56.1)		52.9 (33.4–99.5)	

CI, confidence interval; NR, not reached.

After exclusions of correlated variables, 4 parameters (NLR1, LY3, Ki67 and pathological stage) were included in the multivariate Cox regression analysis (Table 5).

Table 5
Multivariate Cox regression analysis of disease-free and (DFS) and overall survival (OS).

Parameters	HR_{DFS} (95% CI)	*p*	HR_{OS} (95% CI)	*p*
NLR1				
< 2.76	1 (reference)	0.004	1 (reference)	0.002
≥ 2.76	2.6 (1.4–4.8)		2.92 (1.47-5,81)	
LY3				
< 1.55	1 (reference)	0.098	1 (reference)	0.268
≥ 1.55	0.6 (0.3–1.1)		0.66 (0.31–1.38)	
Ki67 (%)				
< 50	1 (reference)	0.008	1 (reference)	0.014
≥ 50	4.1 (1.4–11.4)		3.73 (1.31–10.6)	
Pathological stage				
0-1a	1 (reference)	$3x10^{-4}$	1 (reference)	$7x10^{-5}$
1b-4	4.3 (1.9–9.3)		6.99 (2.68–18.2)	

CI, confidence interval; HR, hazard ratio; LY3, lymphocyte count at the start of the 3rd cycle; NLR1, neutrophil-to-lymphocyte ratio at the start of the 1st cycle.

NLR1, Ki67 and pathological stage proved to be independent markers of DFS (Fig. 1).

The median follow-up was 86.2 (95% CI 82.1-101.2) months. The OS was statistically influenced by similar parameters as were found for DFS except WBC1, LY3, PLR3 and MAI. The 3 independent markers of DFS were also independent markers of OS (Table 5.)

The change in LNR (LNR3-LNR1/LNR1) had no predictive effect on survival (neither on DFS or OS) with a previously reported[15] cut-off level of 0.1258 or a cut-off level calculated by our ROC analysis (data not shown).

We performed further exploratory analysis weather new serologic parameters could give additional predictive information. Patients in the NLR1 < 2.76 group were investigated for further markers and a new variable defined as NLRP = NLR1xNLR3 was evaluated with the cut-off value of 5.84. The DFS and OS curves were significantly (p = 0.002) different according to the dichotomized NLRP (Fig. 2a).

In a similar way patients with NLR1 ≥ 2.76 were investigated for further markers. While NLRP was not significant, another variable defined as PLR = PL1/$(PL3)^2$ with the cut-off level of $2.391x10^{-3}$ was found. The dichotomized PLR resulted in significantly (p = 0.007) different DFS curves (Fig. 2b). PLR was not significant for patients with NLR1 < 2.76.

In the whole cohort of 137 patients 38 patients (28%) reached pCR. Among them the disease relapsed in 9 cases (24%). In the cohort of pCR patients none of the investigated factors were prognostic for DFS or OS.

By analyzing the progression of pCR patients, a new variable was defined: PLP3 = PL3xLY3 with the cut-off level of 631. It was found that the PLP3 level proved to be a promising marker for prediction of relapse after pCR (p = 0.008). All patients who progressed during follow-up had the PLP3 level < 631 (Fig. 2c).

Discussion

Our analysis is in line with previously reported studies showing that markers relatively easily obtained from peripheral blood samples might be important factors for prediction of DFS and OS[17,18]. However, it is important to determine which of the blood markers measured at the beginning of treatment have independent predictive power, and whether consideration of blood parameters during chemotherapy provides more information for this purpose. NLR measured before the start of neoadjuvant chemotherapy (NLR1) was the only independent prognostic serologic marker for prediction of survival. In previous neoadjuvant studies it has not been shown unequivocally that in TNBC NLR1 is an independent factor predicting survival (both DFS and OS) [9,11,14,19,20,21,22,23,24,25]; however, a recent analysis of Bae et colleagues presented on a greater cohort this independent association[13] (Table 6).

Table 6
Prognostic value of baseline neutrophil-to lymphocyte ratio (NLR1) in triple-negative breast cancer (TNBC) patients treated with neoadjuvant chemotherapy.

Reference	N	Neoadjuvant (%)	Adjuvant (%)	significant in MVA	non-significant in MVA
Losada et al.[23]	25	100		(DFS, OS)*	
Asano et al.[24]	61	100			DFS
Goto et al.[25]	83	100			DFS, OS
Chae S. et al.[9]	87	100		pCR	DFS, OS
Patel et al.[11]	126	36.5	52.4	OS	DFS
Our study	137	100		DFS, OS	
Liu C et al.[14]	161	18	81	DFS, OS	
Muñoz-Montaño et al.[20]	261	100		DFS, OS	
Ren K et al.[22]	281	21	79	DFS, OS	
Lee J.et al.[21]	358	14	86	OS	DFS
Qiu X. et al.[19]	406	21.2	78.8	DFS, OS	
Bae SJ et al.[13]	459	100		DFS, OS	
* MVA not performed.					
DFS, disease-free survival; MVA, multivariate analysis; OS, overall survival; pCR, pathologic complete remission.					

It was suggested that incorporating PLR may also be important predicting survival[26,27]. Some reports concluded that the use of SII, which incorporates NLR and PLR, may better predict the efficacy of chemotherapy, compared to either factor separately. This hypothesis has not been investigated specifically in TNBC population treated with neoadjuvant chemotherapy[28,29]. In our analysis PLR and also SII were prognostic in the univariate analysis for DFS and OS, but these associations were less pronounced compared to NLR. In addition, there was a strong correlation with NLR; consequently, these parameters were not included in the multivariate analysis. This observation is consistent with a recent meta-analysis summarizing 39 studies which include also neoadjuvant studies and TNBC patients. This

analysis shows that NLR can predict DFS and OS, but PLR has prognostic role only for OS with less statistical power than NLR[30].

Interestingly, in our analysis Ki67 value and pathological stage had independent prognostic value, but with different cut-offs, than usually applied. Tumors with Ki67 value over 50% had significantly worse survival; however, in clinical practice the 14% or 30% are used as cut-offs[5]. A question is raised whether a 50% cut-off should be applied for TNBC. For cases where pCR was not reached, the relapse rate is much higher and additional chemotherapy is frequently indicated. However, in our study, the survival rate of patients with minimal residual disease (Ia) was similar to that of patients with pCR.This observation reflects the results of Symmans et al[31].

The temporal change and serial measurement during neoadjuvant therapy of these markers may also be important. They could reflect treatment efficacy, change in biological behavior of the tumor or host immune reactions. The prognostic value of markers before the 3rd cycle in general was not as pronounced as NLR at the baseline and did not add prognostic information to baseline values in our analysis. There are data showing that NLR value measured in different time-points during neoadjuvant treatment may have a role in survival prediction[11]. Choi and colleagues reported that change in NLR from baseline to a sample taken shortly before the 3rd cycle ((NLR3-NLR1)/NLR1, with the cut-off level of 0.1258) was predictive for survival[15]. In our analysis this value and a cut-off level calculated by ROC analysis resulted in a non-significant difference of DFS curves (data not shown). The discrepancy can be explained by the fact that the proportion of TNBC patients in the Choi study was only 25%, and as a result, 54% of patients received postoperative hormonal therapy.The blood immune-markers have to be standardized for clinical use. In clinical studies cited in this work the cut-off values of NLR varies between 1.34–4, and PLR varies between 185–292. In the meta-analysis of Guo et al. the cut-off values of NLR under and above 3.0 equally produced significant results[30]. In the case of PLR their results were inconclusive, but PLR above 185 may be more suitable. In our analysis the cut-off for NLR was 2.76 and for PLR 118.4. In ROC analysis the best threshold for NLR3 was 3.27 and for PLR3 225. However, these were not significant factors and not justifiable to use different thresholds at different time points. It seems reasonable to follow the method of Vernieri and colleagues to set the cut-off for NLR at a certain point for clinical purpose and for standardization[32].

In a recent meta-analysis of 52 neoadjuvant studies, pCR rate was 21.1% overall and 30% in TNBC. Pathological complete response was associated with significantly reduced disease recurrence (HR 0.31). This effect was more pronounced in TNBC (HR 0.18). The five-year event-free survival was 88% for those with pCR and was similar with and without adjuvant chemotherapy. The authors stated that attaining pCR after neoadjuvant chemotherapy is likely to reflect tumor biology and highlights the need for further research to evaluate clinical utility of escalation/de-escalation strategies in the adjuvant setting based on neoadjuvant response for patients with localized breast cancer[2]. In our analysis, the pCR rate (28%) was lower than expected which could be explained by the use of older and less effective chemotherapeutic regimes available at that time. The relapse after pCR had no association with NLR or other investigated biomarkers which is in line with the results of Bae et al[13]. The relapse rate after pCR was higher than in the above mentioned meta-analysis which has several causes. It could be partly explained with patient selection. Our center is a national center receiving patients from all regions of Hungary where the medical therapies can vary significantly. In many cases our patients faced problems reaching the oncological centers. Therefore, the OS can be adversely affected by regional differences in access to care. In many cases, the original treatment plan in our cohort was to use chemotherapy both pre- and postoperatively. It is not proven that the same survival results can be achieved by shorter neoadjuvant therapy producing pCR, but without adjuvant treatment than with full chemotherapy. Lastly, no standardized staging was compulsory before starting therapy in that time. However, all these considerations draw attention to the importance of the standardization of oncological care through our country.

Our analysis has some limitations. The data collection took place retrospectively in one institution. The chemotherapy did not follow a uniform scheme, and approximately half of the patients were treated without taxane. Paired data of pathologic prognostic factors in core and surgery specimens were not available in all cases and therefore the change in these factors could not be examined. In this analysis not all previously reported blood markers were considered (e.g. monocyte-to-lymphocyte ratio), although the most relevant parameters found in the literature were included. The tumor infiltrating lymphocyte ratio (TILs) and PD-L1 expression were not measured, though they have predictive and prognostic significance in several investigations and may also have associations with blood immune-markers[21]. Other parameters, which may also influence the OS (e.g. first and further treatment lines of relapsed patients, further surgery or irradiation, etc.) were not recorded.

In this investigation a broad spectrum of characteristics were investigated, among them several common hematological parameters, not just once but at two time-points. From easily accessible putative blood immune-markers NLR at baseline proved to have the strongest prognostic significance. Our search for new blood markers suggests that other markers may also be useful for prognosis prediction beyond wildly accepted prognostic factors such as stage and pCR in triple-negative breast cancer. In our view it is worth exploring the role of such serologic markers also at different time points in a prospective trial, which may help identify patients who potentially benefit from treatment change, de-escalation or escalation.

Methods

Patients

Medical records of consecutive TNBC patients treated preoperatively from 1st of January, 2005 to 31st of December, 2016 were selected from the electronic database of our institution. The investigation had been approved by the Medical Research Council (21679-2/2016/EKU) and the Ethical Committee of the Institute. Informed consent was obtained from all participants. All methods were carried out in accordance with relevant guidelines and regulations. Patients were selected for analysis with TNBC stage II-III who were treated with neoadjuvant chemotherapy (NAC) with or without further adjuvant chemo- and radiotherapy. Patients were excluded from the analysis if they presented other synchronous malignancies (including non-TNBC), or any malignancies in the previous 5 years. Patients who rejected the breast operation were also excluded. The following factors had been considered for analyses: age, body mass index (BMI), cT, cN, c-stage, pathologic features of tumor tissue (histologic grade, tumor-free margin, Ki-67 labeling index, mitotic activity index (MAI), lymphovascular invasion, necrosis), the best response during neoadjuvant treatment, white blood cell count (WBC), NE, LY, PL, NLR, PLR, SII, type of neoadjuvant and adjuvant chemotherapy, type of surgery, pT, pN, pathological stage, completion of chemotherapy, presence of adjuvant radiotherapy. The serologic parameters were registered before the 1st and just before the 3rd cycle of neoadjuvant treatment. The pCR was defined as no invasive tumor in the breast and lymph nodes - ypT0/is and ypN0. The histopathologic characteristics of non-operated patients (stage IV after neoadjuvant treatment) were determined from the biopsy performed before the neoadjuvant treatment.

Statistical analysis

The primary objective was to find predictive markers of the efficacy of NAC beyond preoperative NLR based on LY, NE, PL measured at baseline and before the 3rd cycle of NAC. NAC efficacy measures were characterized by the median disease-free (DFS) and overall survival (OS). The second objective was to discover markers predicting the relapse for patients who achieved pCR. The DFS was calculated from the beginning of neoadjuvant treatment until relapse, second primary, death caused by cancer or the last contact with the patient, whichever occurred first. The OS was

considered from the start of neoadjuvant therapy until the death caused by cancer or last contact with the patient, whichever occurred first. All cut-off values for dichotomization of parameters were calculated by receiver operating curve (ROC) analysis of disease free survival (DFS). The survival curves were analyzed by Kaplan-Meier method and log-rank test was performed. Multivariate Cox regression analysis was also performed and variables which proved to be statistically significant in the univariate analysis were included. Before multivariate analysis the multicollinearity was examined to avoid the inclusion of correlated parameters. Estimates were considered statistically significant if p value was < 0.05. All statistical tests were performed with NCSS 2019 Statistical Software (NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss.).

Declarations

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Authors contribution

All authors contributed to data collection. The data analysis was performed and the first draft of the manuscript was written by GR and BB. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Additional information

The authors declare no competing interests.

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Ethical approval: Medical Research Council (21679-2/2016/EKU) – Hungary (in Hungarian: Egészségügyi Tudományos Tanács, web page: https://ett.aeek.hu/en/secretariat/).

All methods were carried out in accordance with relevant guidelines and regulations.

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Figures

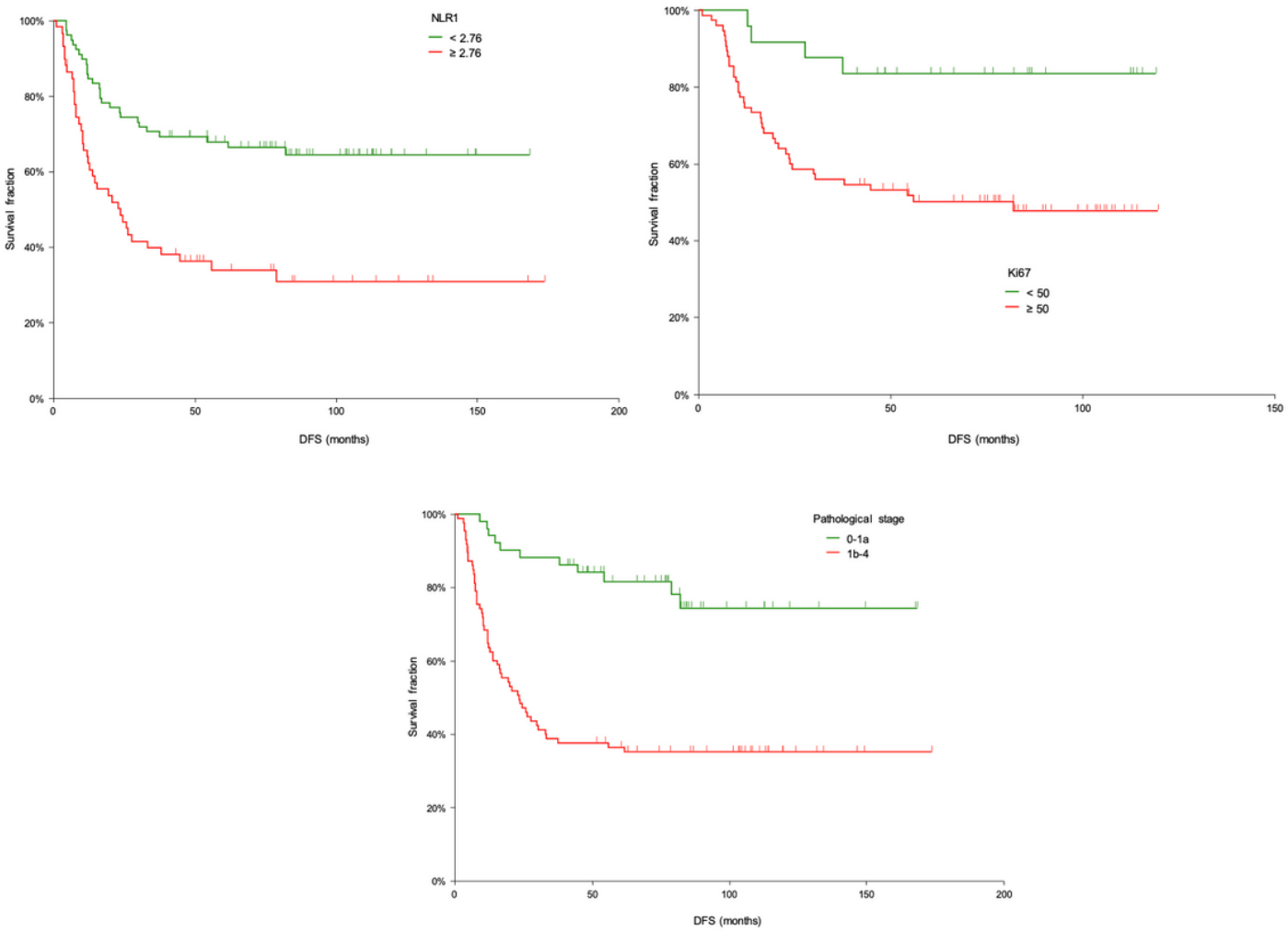

Figure 1

DFS according to NLR1, Ki67, and pathological stage.

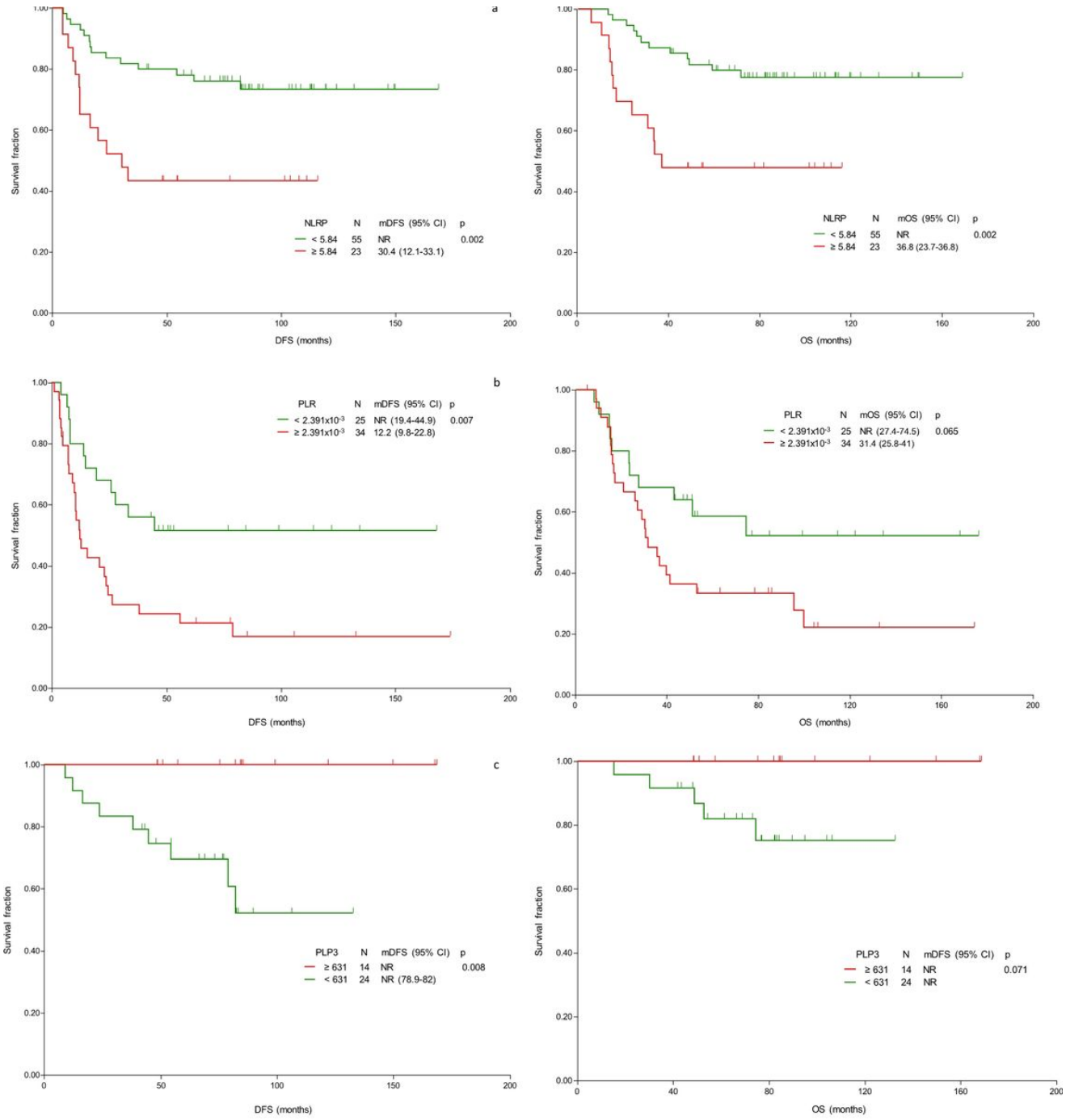

Figure 2

Effect of different exploratory serologic factors on DFS and OS: NLRP (=NLR1xNLR3) in patients with NLR1 < 2.76 (a), PLR (=PL1/PL32) in patients with NLR1 ≥ 2.76 (b); PLP3 (=PL3xLY3) in patients who achieved pCR (c).