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The prognostic value of lymph node response to neoadjuvant therapy among breast cancer subtypes

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ABSTRACT

Objectives. The aim of this study was to assess the prognostic value of persistent node involvement after neoadjuvant chemotherapy among breast cancer subtypes.

Materials and Methods. A total of 258 pacients with T1-T4 and N0-N3 breast cancer treated by neoadjuvant chemotherapy followed by tumor excision and axillary lymph-node dissection between January 2015 and December 2019 were selected from the Coltea Clinical Hospital database and retrospectively evaluated. Association between nodal involvement (ypN) binned into four classes (0, 1-3, 4-9 and ≥10), relapse free-survival and overall survival among the whole population and according to breast cancer subtypes was analyzed using Statistical Package for Social Science Version 29.0.2.0.

Outcomes. After a median follow-up of 20.7 months (range 1-97 months) post neoadjuvant chemotherapy nodal involvement was significantly associated with disease free survival in the whole population ($X^2(3)=23.161$, p <.001) and between breast cancer subgroups ($X^2(3) = 27.871$, p= <.001). After univariate cox regression analyses by breast cancer subtypes nodal involvement was statistically significant only in the Luminal B(HER-) ($X^2(3)=14.867$, p=.002) and triple-negative breast cancer (X2(3)= 9.867, p=.020). In Luminal B(HER2-) breast cancers all nodal involvement subgroups were associated with impaired relapse free survival compared to ypN0 tumors (1-3 nodes, HR= 4.871, 95%CI[1.32-17.94], p=.017; 4-9

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nodes, HR=5.126, 95%CI[1.341-19.59], p=.017; ≥10 nodes, HR=8.744, 95%CI[2.379-32.13], p=.001). In triple negative breast cancers, relapse-free survival was associated with an adverse prognosis in patients with more than 10 nodes involved when compared with ypN0 (HR=16.57, 95%CI[3.25-84.30], p=<.001). There was no statistically significant association in the univariate cox regression analyse between post neoadjuvant chemotherapy nodal involvement and overall survival neither in the whole population ($X^2(3)=.992$, p=.803) nor among breast subtypes ($X^2(3)=1.191$, p= .779). Kaplan Meier analyse of RFS adjusted for BC subtype showed a statistically significant relapse rate in all groups (1-3 (p=.035), 4-9(p=<.001), ≥10 (p=<.001)) compared with ypN0 group. Kaplan Meier overall survival analyse showed no statistical difference in survival among node groups.

Conclusions. Post neoadjuvant chemotherapy lymph node status in breast cancer subtypes represents an important prognostic factor of relapse-free survival and the prognostic value of residual axillary disease should be interpreted according to breast cancer subtype.

Keywords: breast cancer, neoadjuvant chemotherapy, residual axillary disease

Abbreviations: ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; NCCN, National Comprehensive Cancer Network; NAC, neoadjuvant chemotherapy; ER estrogen receptor; PR, progesterone receptore; HER2, human epidermal growth factor receptor 2; BC, breast cancer; RFS, relapse free survival; OS, overall survival; SPSS, Statistical Package for Social Science

INTRODUCTION

Neoadjuvant chemotherapy (NAC) used to be a treatment for patients with locally advanced breast cancer with the primary purpose to reduce tumor size to allow breast-conserving surgery [1,2] . Based on the recognition of that tumor biology rather than anatomic tumor staging is the driver of NAC decisions, currently the role of NAC has expanded to include patients with early-stage, operable breast cancer [1,3,4]. With the continous optimization of chemotherapy regims and the combined use of targeted drugs, NAC increases the rate of tumor downstaiging, allows

treatment response to be clinically assessed (tumor chemosensitivity) and provides evidence for postoperative adjuvant therapy [5-8].

Residual cancer burden (RCB) index incorporates both primary and axillary tumor burden after NAC, reflects chemotherapy responsiveness of a tumor and also predicts patients clinical outcome [3,9,10]. Pathologic complete response (pCR), defined as no residual invasive disease in both the breast an axilla after NAC is a well-known prognostic factor in patients with breast cancer [5,11]. Multiple studies have reported a correlation between breast or axillary pathological complete response and survival [1,12,13]. The aim of this study was to evaluate the prognostic impact of residual axillary burden after preoperative chemotherapy on survival outcomes (RFS, OS) by breast cancer (BC) subtypes

MATERIAL AND METHODS

We analysed 258 patients diagnosed with invasive breast cancer and treated with neoadjuvant chemotherapy at Coltea Clinical Hospital in Bucharest between January 2015 and December 2019. The study was approved by the Ethics Committee of the Coltea Clinical Hospital. After analyzing the availability of biomarkers among the group of pacients only 177 cases could be assigned an intrinsec molecular subtype. Survival analysis were conducted after excluding the missing cases. Neoadjuvant therapy regims were administered based on the recommendation of the National Comprehensive Cancer Network (NCCN) guidelines for breast cancer.

Scoring criteria for ER, PR and HER2 were in accordance with American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) guidelines. Cases were considered estrogen receptor (ER) or progesterone receptore (PR) positive if ≥ 1% of invasive cancer had nuclear staining of any intensity. HER2 expression was considered positive by immunohistochemical score of 3+ and negative by scores of 0 or 1+. Tumors with scores of 2+ were further tested by in situ hybridization (ISH). Index of proliferation Ki-67 was considered high at the threshold value of ≥20%, as advised by the St. Gallen expert panel. Breast cancer subtypes were defined on the basis of the reviewed clinicopathological surrogate definitions at the 13th St. Gallen conference as it follows: luminal A-like, luminal B-like (HER2-), luminal B-like (HER2+), HER2+(non-luminal) and triple-negative. Distinction between

luminal A-like and Luminal B-like (HER2-) was made by a PR positivity ≥20% and a threshold value of ≥20% for Ki-67.

Post-NAC nodal involvement (ypN) was divided into four categories, according to the pathological definition of regional lymph nodes as proposed by AJCC cancer staging manual, 8th edition, namely no axillary involvement (N0), 1 to 3 nodes involved (N 1-3), 4 to 9 (N 4-9) nodes involved and more than 10 (N ≥10) nodes involved.

Residual cancer burden index (RCB) as described by Symmans in 2007 enables the classification of residual disease into four categories: RCB-0 (no residual invasive cancer or pathological complete response), RCB-1 (minimal residual disease), RCB-II (moderate residual disease) and RCB-III (extensive residual disease).

Lymphovascular invasion was defined as the finding of carcinoma in the small vessels outside the main tumor mass (lymphatic or blood vessel).

Statistical Package for Social Science (SPSS) version 29.0.2.0 was used for analysis. The study population was described in terms of frequencies for qualitative variables or medians and means for quantitative variables. Differences in categorical variables were analyzed using Chi-square test of homogeneity or Fisher Exact Test with post hoc anlysis and differences in continous variables were evaluated using Kruskal-Wallis H test. Differences were considered significant for p-values ≤ 0.05 with Bonferroni correction when required. Relapse free survival (RFS) was defined as the time from surgery to the time of local or distant recurrence and overall survival was defined as the time from surgery to death. Cox regression analysis was used to estimate de hazard ratios and their 95% confidence interval (CI). A two-sided p-value of ≤ 0.05 was considered statistically significant. Survival curves were plotted using Kaplan-Meier method and compared using the log-rank test.

Outcomes

A total of 258 patients were inlcuded in this study. Patients characteristics are summerized in Table 1. Median age in the whole population was 61 years old (mean age 59.46). At diagnosis 92.6% patients were node positive and 7.4% node negative. After NAC 34.5% patients were ypN0 and 65.5% ypN positive. Patients repartition by breast cancer subtype was as it follows: 37 (20.9%) patients were luminal A, 81(45.8%) were luminal B(HER2-), 15(8.15%) patients were luminal B(HER2+), 13(7.3%) patients were HER2(non-luminal) and 31(17.5%) patients were triple

negative. Repartition of node negative patients at diagnosis among breast cancer subtypes was as it follows: 6.3%(1) were luminal A, 50%(8) were luminal B(HER2-), 6.3%(1) were luminal B(HER2+), 6.3%(1) were HER2+(non-luminal) and 31.3%(5) were triple negative. Repartition of node positive patients at diagnosis among breast cancer subtypes was as it follows: 22.4%(36) Luminal A, 45.3%(73) Luminal B(HER2-), 8.7%(14) Luminal B(HER2+), 7.5%(12) HER2+, 16.1%(26) triple negative, ($X^2(4)= 3.759$, p= .389).

After neoadjuvant chemotherapy among node positive patients 21.1%(24) had luminal A breast cancer, 52.6%(60) had luminal B (HER2-), 7%(8) had luminal B(HER2+), 4.4% (5) had HER2 (non-luminal) and 14.9%(17) had TNBC. At NAC completion were mole likely to have a nodal involvement patients with following characteristics at diagnosis: cT3-T4 tumors, that were positive for ER and PR, negative for HER2 and Luminal B(HER2-) subtype. The axilla pthologic complete response (ypN0) was more frecquent in cT1-T2 tumors, who had a Ki-67 proliferation index more than 20% and that were intermediate histological grade.

Table 1. Patients and tumor characteristics by post- neoadjuvant chemotherapy (NAC) nodal involvement

Characteristics	Class	All cases	Node negative	Node positive	
n		**258 (100%)**	**89 (34.5%)**	**169 (65.5%)**	***p* value**
Median age		61y(59.46y)	58y(57.90y)	61y(60.28y)	.860
Age groups	0-50	61(23.6)	27(30.3)	34(20.1)	.156
	50-60	66(25.6)	19(21.3)	47(27.8)	
	60+	131(50.8)	43(48.3)	88(52.1)	
Menopausal status	Premenopausal	59(22.9)	25(28.1)	34(20.1)	.147
	Postmenopausal	199(77.1)	64(71.9)	135(79.9)	
BMI	<18.5	2(1.1)	1(1.6)	1(0.8)	.870
	18.5-24.9	39(20.7)	14(22.2)	25(20)	
	25-29.9	67(35.6)	23(36.5)	44(35.2)	
	≥30	80(42.6)	25(39.7)	55(44)	
Clinical T	T1-T2	129(50)	56(66.3)	70(41.4)	<.001

	T3-T4	129(50)	30(33.7)	99(58.6)	
Clinical N	N0	19(7.4)	12(13.5)	7(4.1)	.006
	N1-N2-N3	239(92.6)	77(86.5)	162(95.9)	
ER status	Negative	44(25)	22(36.1)	22(19.1)	.036*
	1-10%	8(4.5)	3(4.9)	5(4.3)	
	>10%	124(70.5)	36(59)	88(76.5)	
PR status	Negative	59(33.5)	26(42.6)	33(28.7)	.166
	<20%	34(19.3)	11(18)	23(20)	
	≥20%	83(47.2)	24(39.3)	59(51.3)	
HER2 status	Negative	149(84.7)	47(77)	102(88.7)	.041
	Positive	27(15.3)	14(23)	13(11.3)	
Ki-67	<14%	30(17.3)	11(18)	19(17)	.968
	14-19%	24(13.9)	8(13.1)	16(14.3)	
	≥20%	119(68.8)	42(68.9)	77(68.8)	
Histological type	NST	213(82.6)	78(87.6)	135(79.9)	.553
	Lobular	35(13.6)	9(10.1)	26(15.4)	
	Metaplastic	5(1.9)	1(1.1)	4(2.4)	
	other	5(1.9)	1(1.1)	4(2.4)	
Tumoral grade	I	42(16.3)	15(16.9)	27(16)	.372
	II	172(66.7)	55(61.8)	117(69.2)	
	III	44(17.1)	19(21.3)	25(14.8)	
DCIS Component	absent	164(63.6)	59(66.3)	105(62.1)	.509
	present	94(36.4)	30(33.7)	64(37.9)	
LVI	absent	201(77.9)	82(92.1)	119(70.4)	<.001
	present	57(22.1)	7(7.9)	50((29.6)	
BC subtype	Luminal A	37(20.9)	13(20.6)	24(21.1)	.054
	Luminal B(HER2-)	81(45.8)	21(33.3)	60(52.6)	
	Luminal B(HER2+)	15(8.15)	7(11.1)	8(7)	
	HER2+ (non-Luminal)	13(7.3)	8(12.7)	5(4.4)	
	TNBC	31(17.5)	14(22.2)	17(14.9)	

Abbreviations: BMI = body mass index; T= tumor; N= node; ER= estrogen receptor; PR= progesteron receptor; HER2= human epidermal growth factor receptor 2; NST=no special type; DCIS= ductal

carcinoma in situ; LVI= lymphovascular invasion; BC=breast cancer; TNBC=triple negative breast cancer. Missing data: BC subtypes, n=81; ER, n=82; PR, n=82, Ki-67, n=85. *Post hoc analysis involved pairwise comparisons using multiple Fisher's exact test with a Bonferroni correction. Statistical significance was accepted at p < .016667.

The number of removed nodes varied from 1 to 36 with a median of 16 (mean 16.23) (Figure 1A) and the number of lymph nodes involved ranged from 0 to 33 with a median of 2 (mean 4.28) (Figure 1B). A Kruskal-Wallis H test was run to determine if there were differences in removed nodes scores between the five breast cancer subtypes. As assessed by visual inspection of a boxplot, distributions of removed nodes were not similar for all groups, but the mean rank was not statistically significant between groups, $X^2(4)= 5.960$, p=.202. The lowest score of removed nodes was observed in HER2 amplified cases (HER2+, 75.77; Luminal B(HER2+),76.11) folowed by TNBC (80.53), Luminal B(HER2-) (86.53) and Luminal A (103.85). For involved nodes, visual inspection of the boxplot (Figure 1.3.) showed that the distributions of involved nodes scores were not similar but also were not statistically significat between groups, $X^2(4) = 8.722$, p=.068. The nodal involvement scores decreased from Luminal B(HER2-)(99.10), to Luminal A (89.32), to Luminal B(HER2+) (83.63), to TNBC (73.52) to HER2+(non-luminal) (68.23).
After NAC nodal involvement in the whole population was as it follows: 34.5%(89) ypN0, 31%(80) ypN 1-3, 18.2%(47) ypN 4-9 and 16.3%(42) ypN ≥10 nodes.

Figure 1.1.

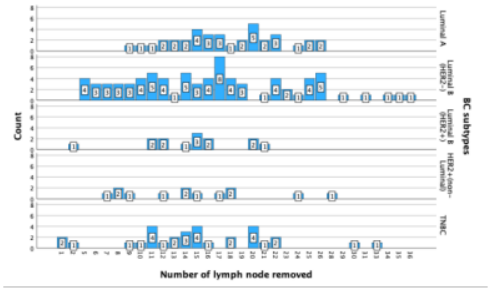


Figure 1.2.

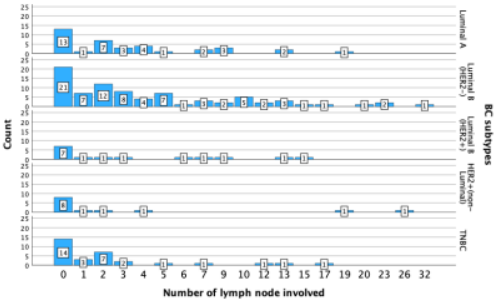


Figure 1.3.

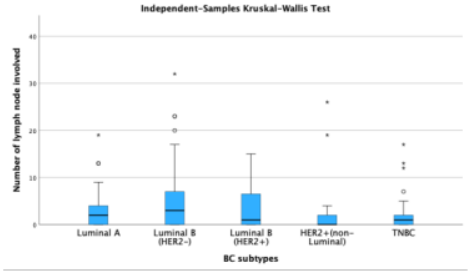


Figure 1.4.

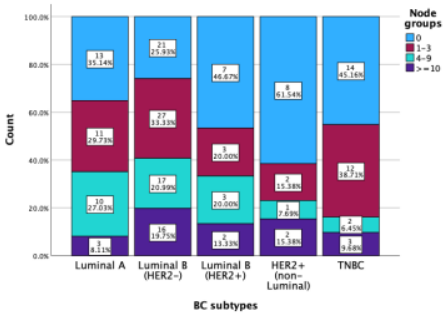


Figure 1. Nodal involvement after NAC according to BC subtype: **(1)** number of removed lymph nodes; **(2)** number of involved lymph nodes; **(3)** mean number of involved lymph nodes; **(4)** node involvement repartition according to BC subtype.

Post-NAC tumor characteristics according breast cancer subtypes are summerized in Table 2. Association between BC subtypes and treatment response categories (pCR, pPR, NR) was statistically significant, $X^2(4)=15.921$, p=.003. Only 6.2%(11) cases had a pCR and the highest rate was observed in the HER2+ subgroup. Distribution of RCB rates showed a statistical significant difference, $X^2(4)= 11.603$, p=.021 as it follows: HER2+ showed a statistical significant difference compared with Luminal A (p=.011), with HER2+ having the highest percent of RCB-0, and compared with Luminal B(HER2-), the latter showing the highest rate of extensive residual disease, p=.003.

Table 2. Post-neoadjuvant chemotherapy tumor characteristics by breast cancer subtype

Characteristics		Luminal A	Luminal B (HER2-)	Luminal B (HER2+)	HER2+	TNBC	*p*
Class	**n(%)**						
pCR	11(6.2)	0(0)	3(27.3)	1(9.1)	5(54.5%)	1(9.1)	.003
pPR	147(83.1)	33(22.4)	67(45.6)	13(8.8)	6(4.1)	28(19)	
NR	19(10.7)	4(21.1)	11(57.9)	1(5.3)	1(5.3)	2(10.5)	
RCB-0	11(6.2)	0(0)	3(27.3)	1(9.1)	6(54.5)	1(9.1)	.021
RCB-I	42(23.7)	9(21.4)	15(35.7)	6(14.3)	2(4.8)	10(23.8)	
RCB-II	88(49.7)	21(23.9)	43(48.9)	6(6.8)	3(3.4)	15(17)	
RCB-III	36(20.3)	7(19.4)	20(55.6)	2(5.6)	2(5.6)	5(13.9)	
ypN0	63(35.6)	13(20.6)	21(33.3)	7(11.1)	8(12.7)	14(22.2)	.065

ypN 1-3	55(31.1)	11(20)	27(49.1)	3(5.5)	2(3.6)	12(21.8)
ypN 4-9	33(18.6)	10(30.3)	17(51.5)	3(9.1)	1(3)	2(6.1)
ypN ≥10	26(14.7)	3(11.5)	16(61.5)	2(7.7)	2(7.7)	3(11.5)

Abbreviations: pCR = pathological complete response; pPR=pathological partial response; NR=no response; RCB=residual cancer burden; N=node

During the follow-up time (range, 1-96 months), 62(24%) of 258 patients had experienced relapse and 8 (3.1%) of 258 patients had died. The median follow-up for all patients was 20.76 months and between subgroups was as it follows: for Luminal A patients was 27.41 months, for Luminal B(HER2-) was 25.63 months, for Luminal B (HER2+) was 34.07 months, for HER2(non Luminal) was 24.15 and for TNBC was 29.55 months. In the univariate analysis among whole population, were significantly associated with RFS the clinical tumor size, the clinical nodal status, ER and PR status, index of proliferation Ki-67, histological type, LVI, breast cancer subtype, the pathological response to NAC therapy, the pathological nodal involvement and RCB assessment (Table 3). In the multivariate analysis statistical significance showed LVI, clinical T and N and ER status (Tables 3, 4).

Table 3. Association of clinical and pathological pre- and post-neoadjuvant chemotherapy parameters with relapse-free survival after univariate analysis in the whole population

				Univariate		
Variable	**Category**	***n***	**Events**	**HR**	**95% CI**	***p***
Age groups	[0-50) vs.	61	10			**.314***
	[51-69)	66	21	1.748	[.822 - 3.721]	.147
	60+	131	31	1.524	[.746 - 3.115]	.247
Menopausal status	Pre- vs. postmenopausal	59	10			**.185***
		199	52	1.549	[.786 - 3.056]	
BMI	<18.5 vs.	2	1			**.967***
	18.5-24.9	39	12	1.154	[.149 - 8.920]	.891
	25-29.9	67	15	1.123	[.147 - 8.563]	.911
	≥30	80	25	1.296	[.175 - 9.627]	.800
Clinical T	T1-T2 vs.	129	17			**<.001***

	T3-T4	129	45	3.724	[2.116 - 6.556]	
Clinical N	N0-N1 vs.	168	27			
	N2-N3	90	35	2.557	[1.546 - 4.227]	**<.001***
ER status	Negative vs.	44	21			**.070***
	1-10%	8	3	.627	[.186 - 2.120]	.453
	>10%	124	35	.516	[.300 - . 888]	.017
	Negative vs.	44	21			**.022***
	positive	132	38	.524	[.307 - .894]	
PR status	Negativ vs.	59	27			**.004***
	<20%	34	16	.934	[.503 - 1.734]	.828
	≥20%	83	16	.384	[.206 - .714]	.002
	Negative vs.	59	27			**.023***
	positive	117	32	.545	[.326 - .912]	
HER2 status	Negative vs.	149	51			**.544***
	positive	27	8	.796	[.374 - 1.696]	
Ki-67	<14% vs.	30	4			**.003***
	14-19%	24	5	1.346	[.360 - 5.022]	.659
	≥20%	119	50	3.459	[1.248 - 9.591]	.017
Histological	NST vs.	213	43			**<.001***
type	Lobular	35	14	1.879	[1.023 - 3.449]	.042
	Metaplastic	5	5	9.792	[3.742 - 25.622]	<.001
	other	5	0	.000	[.000 - 5.540]	.968
Tumoral						
grade	I vs.	42	8			**.677***
	II	172	39	1.194	[.557 - 2.560]	.648

	III	44	15	1.455	[.612 - 3.458]	.396
DCIS status	negative vs.	164	40			**.778***
	positive	94	22	.928	[.551 - 1.562]	
LVI	negative vs.	201	34			**<.001***
	positive	57	28	3.707	[2.226 - 6.173]	
BC subtype	Luminal A vs.	37	2			**<.001***
	Luminal B(HER2-)	81	32	8.811	[2.107 - 36.842]	.003
	Luminal B(HER2+)	15	4	4.783	[.867 - 26.372]	.072
	HER2+(non-Luminal)	13	4	7.144	[1.308 - 39.032]	.023
	TNBC	31	17	12.904	[2.971 - 56.043]	<.001
Pathological response	pCR vs.	11	2			**.001***
	pPR	215	45	1.056	[.256 - 4.360]	.940
	NR	32	15	3.616	[.825 - 15.855]	.088
RCB	RCB-0 vs.	11	2			**<.001***
	RCB-I	59	7	.573	[.119 - 2.759]	.487
	RCB-II	126	30	1.070	[.255 - 4.491]	.927
	RCB-III	62	23	3.538	[.829 - 15.105]	.088
ypN	0 ggl. vs.	89	11			**<.001***
	1-3 ggl.	80	18	2.066	[.974 - 4.383]	.059
	4-9 ggl.	47	15	2.603	[1.194 - 5.673]	.016
	≥10	42	18	6.215	[2.904 - 13.297]	<.001

Abbreviations: BMI = body mass index; T= tumor; N= node; ER= estrogen receptor; PR= progesteron receptor; HER2= human epidermal growth factor receptor 2; NST=no special type; DCIS= ductal carcinoma in situ; LVI= lymphovascular invasion; BC=breast cancer; TNBC=triple negative breast cancer; pCR = pathological complete response; pPR=pathological partial response; NR=no response; RCB=residual cancer burden

After univariate analysis post-NAC nodal involvement was statistically associated with RFS in the whole population, $X^2(3)=23.161$, p <.001 (Table 3). After analyses by breast cancer subtype, the association between nodal involvement binned by 4 classes and RFS was significantly different between BC subgroups, $X^2(3) = 27.871$, p= <.001, but at variance within groups (Figure 1A). Patients having between 4-9 and more than 10 nodes involved were associated with impaired RFS after univariate analysis, HR=2.60, 95% CI [1.19-5.67] and HR= 6.21, 95% CI [2.90-13.29]. In the multivariate analysis de nodal involvement in the whole population was not statistically significant, p= .168 (Table 4).

Table 4. Association of clinical and pathological pre- and post-neoadjuvant chemotherapy parameters with relapse-free survival after multivariate analysis in the whole population

					Multivariate	
Variable	**Category**	***n***	**Events**	**HR**	**95% CI**	***p***
Clinical T	T1-T2 vs.	129	17			**.003**
	T3-T4	129	45	3.282	1.499 - 7.186	
Clinical N	N0-N1 vs.	168	27			**.029**
	N2-N3	90	35	2.162	1.082- 4.319	
ER status	Negative vs.	44	21			**.015**
	positive	132	38	.070	.008 - .600	
PR status	Negativ vs.	59	27			**.043**
	<20%	34	16	3.480	1.066 - 11.364	.039
	≥20%	83	16	1.415	.459 - 4.363	.546
Ki-67	<14% vs.	30	4			**.603**
	14-19%	24	5	.471	.085 - 2.610	.388
	≥20%	119	50	.849	.219 - 3.291	.812
Histological	NST vs.	213	43			**.182**
type	Lobular	35	14	1.832	.913 - 3.678	.089
	Metaplastic	5	5	3.198	.721 - 14.192	.126

	other	5	0	.000	.000 - 2.262E	.971
LVI	negative vs.	201	34			**.028**
	positive	57	28	2.084	1.082 - 4.016	
BC subtype	Luminal A vs.	37	2			**.357 (3[a])**
	Luminal B(HER2-)	81	32			
	Luminal B(HER2+)	15	4	4.107	.693 - 24.320	.120
	HER2+(non-Luminal)	13	4	3.826	.535 - 27.369	.181
	TNBC	31	17	.597	.174 - 2.054	.414
Pathological response	pCR vs.	11	2			**.902**
	pPR	215	45	1.365	.178 - 10.451	.764
	NR	32	15	1.547	.203 - 11.791	.674
RCB	RCB-0 vs.	11	2			**.880 (2[a])**
	RCB-I	59	7			
	RCB-II	126	30	.932	.216 - 4.029	.925
	RCB-III	62	23	.827	.362 - 1.888	.652
ypN	0 nodes vs.	89	11			**.168**
	1-3 nodes	80	18	1.669	.556 - 5.015	.361
	4-9 nodes	47	15	1.304	.369 - 4.600	.680
	≥10 nodes	42	18	2.965	.855 - 10.278	.087

a. Degree of freedom reduced because of constant or linearly dependent covariates

Abbreviations: T= tumor; N= node; ER= estrogen receptor; PR= progesteron receptor; HER2= human epidermal growth factor receptor 2; NST=no special type; DCIS= ductal carcinoma in situ; LVI= lymphovascular invasion; BC=breast cancer; TNBC=triple negative breast cancer; pCR = pathological complete response; pPR=pathological partial response; NR=no response; RCB=residual cancer burden

In Luminal A ($X^2(3)=4.669$, p= .198) and Luminal B(HER2+) ($X^2(3)=3.624$, p= .305) the post NAC nodal involvement showed no statisticaly significant difference within the groups (Fig. 2.2, 2.4). The omnibus tests of model coefficients showed a statistically significant difference by the HER2(non-luminal) type, $X^2(3)= 9.731$, p=.021, but comparison between categories of nodal involvement showed no statistical difference. In the Luminal B(HER2-) there was found a statistically

difference between all nodal involvement subgroups compared to N0, $X^2(3)=14.867$, p=.002. In the triple negative subgroup patients with high nodal involvement (≥ 10 nodes) were associated with an adverse prognosis, HR=16.573, 95% CI [3.258 – 84.307], X2(3)= 9.867, p=.020.

Table 5. Association between relapse free survival and residual axillary burden after neoadjuvant chemotherapy according to breast cancer subtype, univariate analysis.

Variable	Category	*n*	Events	HR	95% CI	*p*
Luminal A	0 vs.	13	0			**.815**
	1-3	11	0	1.00	.000	1.00
	4-9	10	1	100336.6	.000-2.62E	.970
	≥10	3	1	396993.0	.000-1.03E	.967
Luminal B (HER2-)	0 vs.	21	3			**.013**
	1-3	27	10	4.871	1.322-17.944	.017
	4-9	17	8	5.126	1.341-19.595	.017
	≥10	16	11	8.744	2.379-32.135	.001
Luminal B (HER2+)	0 vs.	7	1			**.846**
	1-3	3	0	.000	.000	.986
	4-9	3	2	1.331	.082-21.477	.840
	≥10	2	1	3.428	.207-56.770	.390
HER2+(non-luminal)	0 vs.	8	2			**.875**
	1-3	2	0	.033	.000-282.50	.997
	4-9	1	0	.693	.000-1.132E	.997
	≥10	2	2	3979.77	.000-3.081E	.709
TNBC	0 vs.	14	5			**.009**
	1-3	12	7	2.333	.738-7.378	.149
	4-9	2	2	2.951	.548-15.885	.208
	≥10	3	3	16.573	3.258-84.307	<.001

Abbreviations: HER2=human epidermal growth factor receptor 2; TNBC=triple negative breast cancer

Figure 2.1.

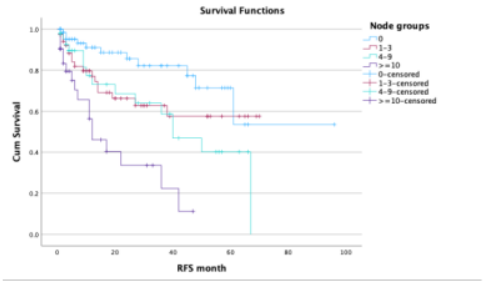


Figure 2.2.

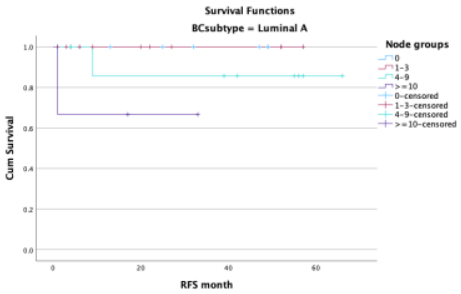
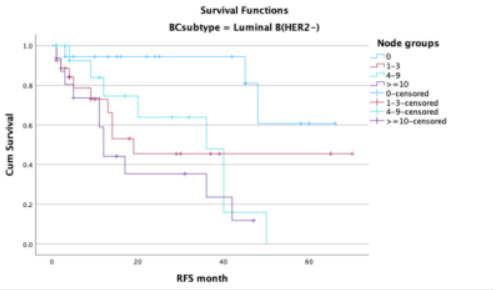


Figure 2.3.



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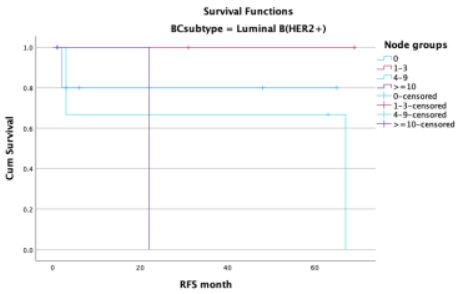

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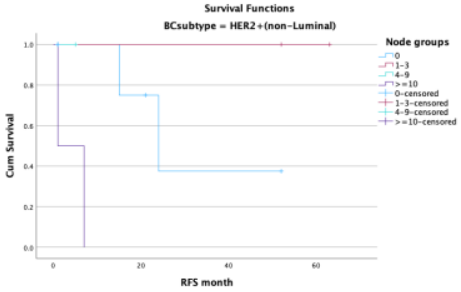

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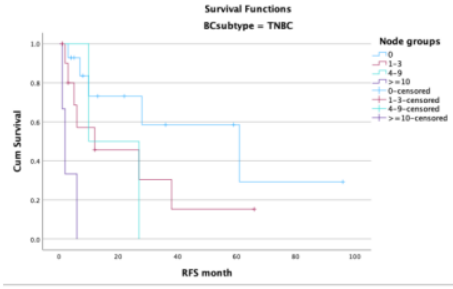

Figure 2. Relapse-free survival according in the whole population (1), in Luminal A breast cancer (2), in Luminal B (HER2-) breast cancer (3), in Luminal B (HER2+) breast cancer (4), in HER2 (non-luminal) breast cancer (5), in triple negative breast cancer (6).

Post-NAC nodal involvement in the univariate cox regression was not significantly associated with OS neither in the whole population ($X^2(3)=.992$, p=.803) nor after analyses by BC subtypes ($X^2(3)=1.191$, p= .779).

DISCUSSION

Prognosis of breast cancer patients receiving NAC ist associated with tumor biology and tumor response to chemotherapy in the breast and axilla [5]. The potential of neoadjuvant systemic therapy to reduce the extent of breast surgery is well established but its potential to reduce axillary surgery is less well evaluated [14]. The management of the axilla after neoadjuvant chemotherapy is still dominated by axillary lymph node dissection (ALND) in order to establish nodal status and guide adjuvant treatment indication [1,14,15] . Currently lymph node status remains an important factor in the AJCC prognostic staging and remains an essential determinant of adjuvant treatment decision-making [16,17]. In order to accurately stage the axilla, the NCCN defines an adequate axillary lymph node dissection the retrieval of at least 10 lymph nodes [18,19]. Rosenberger LH et al. found that fewer dissected lymph nodes were associated with poorer OS in breast cancer with positive lymph nodes [18,19]. Previous studies reported that neoadjuvant chemotherapy can induce histomorphological changes within lymph nodes and thus may lead to decreased lymph node harvest rates [19-22]. A retrospective study using data from the National Cancer Database demonstrated that the yield of axillary lymph node dissection was significantly lower in patients who received NAC than those who underwent surgery alone [19,23-25].

There is an interest in performing de-escalating axillary surgery after neoadjuvant chemotherapy [1]. Axillary lymph node dissection comes with many postoperative complications such as lymphedema, range-of-motion restrictions, arm paresthesia and pain [1,26]. Therefore increasing efforts are made to investigate the feaibility of less morbid sentinel lymph node biopsy (SLNB) before or after NAC [1]. The ACOSOG Z0011 phaze 3 randomized clinical trial determined that, among women with T1 or T2 invasive primary breast cancer, no palpable axillary adenopathy and 1 or 2 sentinel lymph nodes containing metastases, the 10-year overall survival for patients treated with SLND alone was not inferior to overall survival of those treated with axillary lymph node dissection [1,27,28]. SLNB is known as the standard of care

for axillary node staiging of patients with early breast cancer and its necessity was questioned since surgery for examination of axillary nodes is not performed with curative intent [29]. The objective of the SOUND randomomized clinical trial was to adress this question and to determine whether the omission of axillary surgery was noninferior to SLNB in patients with small BC and a negative result on preoperative axillary lymph node ultrasonography [1,29]. The findings of the SOUND trial demonstrated that patients who did not undergo axillary surgery had noninferior 5-year distant disease-free survival compared with those who underwent sentinel lymph node biopsy, findings suggesting that patients with a BC of a diameter equal to or smaller than 2 cm and a negative result on preoperative axillary lymph node ultrasonography can be safely spared of any axillary surgery whenever the lack of pathological information does not affect the postoperative treatment plan [1,29]. The ongoing EUBREAST1 and ASICS trials aim to determine whether axillary surgery can be abandoned in selected patients receiving NAC before surgery [1,30,31]. On the other hand, it is sustained the necesity of surgical axillary staging after NAC through the pathological nodal status significant prognostic value [1,32,33].
It has been reported that the rate of axillary downstaging after neoadjuvant chemotheray ranges as widely as 20 to 60% and can be up to 64.7% in selected subtypes such as HER2 positive cases [14,34]. In our study on cN positive patients, the percentage of pathologic complete respone in axilla after NAC was 34.5%. Several studies evaluated the association of pathological complete response in axilla with a clinical node status before NST. It is reported that the rate of involved nodes after NAC was 2-22% in cN0 patients [35,36,37], 34-59% in cN1 patients [37,38] and 20-61% depending on breast cancer subtype in cN positive patients [14, 39,40]]. In our study the rate of involved nodes was 36.8% in cN0 patients and 67.8% in cN1-N3 and between 21-54% depending on the subtype. The observed rates of downstaging by breast cancer subtype was as it follows: HER2+(non-luminal) had 58.3% rate, Luminal B (HER2+) registered a 42.9% rate, TNBC a 38.5% rate, Luminal A (36.1%) and Luminal B(HER2-) registered a rate of 21.9%. It is generally accepted that achieving a pCR after neoadjuvant chemotherapy is associated with an improved prognosis over all tumor molecular subtypes [34]. Trials have reported that the association between pathological complete response in the axillary nodes and prognosis is stronger than the influence of breast pathologic complete response [34]. In our study the highest rate of pCR (both breast and axilla)

was observed in HER2+(non-luminal) with 54.5% rate, followed by Luminal B (HER2-) with a perecentage of 27.3%. TNBC and Luminal B (HER2+) had a equal rate of 9.1% and Luminal A registered no pCR. The highest rates of no response (NR) were observed in Luminal B(HER2-) (57.9%) and Luminal A (21.1%). The highest rates of residual cancer (RCB-III) were observed in Luminal B(HER2-) (55.6%) and Luminal A (19.4%). Despite the highest rates of downstaiging observed in the HER2 amplified and TN breast cancer subtypes the highest ypN0 frequences in the whole population were observed in Luminal B(HER2-) subgruoup and the distribution of ypN0 was as it follows: 20.6% luminal A, 33.3% Luminal B (HER2-), 11.1% Luminal B(HER2+), 12.7% HER2+ and 22.2% TNBC. However, the lowest rates of ypN positive had HER2 amplified subtypes (Luminal B (HER2+) (7%) and HER2+(non-luminal) (4.4%)) folowed by TNBC (14.9%). Luminal B (HER2-) had a 52.6% rate of ypN positive and Luminal A of 21.1%. A statistical signficant association with ypN negative was seen in the clinical tumor size (66.3% were cT1-T2 tumors) and LVI (92.1% were LVI negative). Among ypN negative cases, grade 2 tumors (61.8%) and values ≥20% of Ki-67 (68.9%) were most frequently encountered, though without statistically significance compared with ypN positive. Among characteristics of the tumors, the ones who achieved a pCR were grade II (72.7%), ER negative (63.6%, $X^2(2)=8.668$, p=<.010), PR negative (63.6%), HER2+ positive (63.6%, $X^2(2)=14.828$, p=<.001), had a Ki-67 ≥20%, were ER-/HER2+ (54.5%, p= <.001) and HER2+(non-luminal) (54.5%, p= .001). Compared with other studies, in our study the higher rates of total pCR (both axila and breast) were observed in HER2 positive tumors and TNBC however these where not observed in the pathological complete response of the axilla.

At the completion of a total of 96 months follow up time in the whole population, the ypN0 group had percentage of censored cases of 87.6%, group ypN 1-3 of 77.5% cases, ypN 4-9 group of 68.1% cases and ypN ≥10 of 57.1% cases. Patients in the ypN0 group had a mean time to relapse at 69.81 months (95% CI, 55.49 to 84.13 months). This was longer that ypN ≥ 10 group with a 19.89 months (95% CI, 12.87 to 26.91 months) mean time to relapse (p= < .001) and ypN 4-9 group with 40.94 months (95% CI, 30.75 to 51.12) mean time to relapse (p= .017). Group ypN 1-3 had a mean time to relapse of 45.97 months (95% CI, 37.03 to 54.90) but showed no statistical difference compared with ypN0 group (p= .052). Kaplan Meier pairwise

comparison of yp nodes subgroups adjusted for BC subtype showed a statistical significance among all groups including ypN 1-3 compared with ypN 0 ($X^2(3)= 4.453$, p=.035). Among breast cancer subtypes the highest mean time to replase had the luminal A subtype (61.83 months, 95% CI, 56.17 to 67.49 months). The lowest mean time to relapse had TNBC (34.26 months, 95% CI, 19.02 to 49.50, p=<.001), followed by Luminal B(HER2-) (37.08 months, 95% CI, 29.816 to 44.35, p=<.001) and HER2+ (non-luminal) (38.08 months, 95% CI, 20.04 to 56.12, p=.001). Luminal B (HER2+) had a mean time to relapse of 50.71 months (95% CI, 34.08 to 67.34) and when compared with Luminal A showed no statistical difference (p=.088).
A log rank test was run to determine if there were differences in the overall survival distribution for the four nodes subgroups in the whole population and adjusted for BC subtype and both survival distributions were not statisticaly different, $X^2(3)= 1.163$, p=.762. The distribution of the 8 cases who died among the node subgroups was as it follows: two were in ypN0 subgroup, three were in the ypN 1-3, one was in the ypN 4-9 and two were in the ypN ≥10 groups. According to the BC subtype, 3/8 patients who died were in the luminal B (HER2-) BC subgroup and 5/8 cases were TNBC.

CONCLUSIONS

The study has some limitations, firstly the study sample is small and secondly the follow-up time is too short. Lymph node status after NAC represents an important prognostic factor of relapse-free survival in breast cancer subtypes. Discrepancy between rates of breast pathologic complete response, axillary node response and total pathologic complete response (breast and axilla) and their impact on survival outcomes in different intrinsic subtypes of brest cancer after neoadjuvant chemotherapy should be further investigated in order to accurately stratify patients with a high risk of recurrence and to asses the posibility of de-escalation of axillary surgery.

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