Protein Biomarker Expression in Invasive Breast Carcinoma, NST Classified According to Age, TNM and Original Nottingham Prognostic Index – A Study in Kosovo

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Abstract

Prognosis and management of patients with invasive breast carcinoma, NST is dependant on a few established parameters such as: TNM stage, Nottingham Prognostic Index, age, Estrogen and Progesterone Receptor expression, Her-2/neu amplification, proliferation index as measured by Ki-67 and molecular profiling.

In our study we analyzed immunohistochemical expression of Estrogen Receptor, Progesterone Receptor, Her-2/neu, Ki-67 and p53 in in patients classified according to Nottingham Prognostic Index, TNM stage and age.

In patients classified according to the original Nottingham Prognostic Index there was significant difference in biomarker expression. In contrast, this difference was not observed when patients were classified according to TNM. In analysis of marker expression in different age groups, significant difference was observed only with p53, expression of which was seen more frequently in younger age group.

In conclusion, prognostic stratification of patients by the original Nottingham Prognostic Index is related to expression of well-established biomarkers of breast cancer. p53 showed to be the only biomarker with significantly higher expression in younger patients.

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Introduction

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Histologic grading by Nottingham is a rigorous modification of Elston and Ellis criteria, which in turn, are a modification of Scarff-Bloom-Richardson system. This system is based on three important histological parameters such as a). level of tubule formation, scored as 1-3 (1=more than 75% tubule formation, 2=10-75% tubule formation and 3=less than 10% tubule formation by the tumor cells), b). nuclear atypia/polymorphism scored as 1 to 3 and c). mitotic activity scored 1 to 3 (1=less than 8/10HPF, 2=8 to 15/10HPF and 3=more than15/10HPF). Histological grade is subsequently expressed as a score of 1 to 3 which is determined by adding the values of the individual parameter. Values 3 to 5 are scored as grade 1, values 6 to 7 are scored as grade 2 and values 8 to 9 are scored as grade 3.[2-9]

Nottingham Prognostic Index (NPI) is a numerical value calculated by adding the values of a).tumor size expressed in cm and multiplied by a coefficient of 0.2, b). number of positive lymph nodes (1=negative lymph nodes, 2=one-to-three positive lymph nodes and 3=more than three positive lymph nodes), and c). histological grade (1,2 or 3). According to this system the patients are stratified in three prognostic groups, namely a). good prognostic group, with a value of

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≤3.4, b). moderate prognostic group, with a value of 3.4-5.4 and, c). poor prognostic group, with a value above 5.4.[9]

Besides NPI, it is generally accepted that young age is also an independent prognostic indicator. Less than 10% of women with breast cancer are diagnosed before the age of 40 years. Survival rates are worse when compared to those in older women, and multivariate analysis has shown younger age to be an independent predictor of adverse outcome.[10]

Material and methods

Paraffin blocks from patients diagnosed with invasive breast carcinoma, NST were retrieved from our archive. The cases included in our study were female patients who were treated with radical mastectomy and axillary dissection. The cases had been previously staged according to the pathologic tumor-node-metastasis (pTNM) system and appointed histological grade according to Nottingham criteria (Table 1).

NPI	
Value	*Prognosis*
≤3.4	good
3.41-5.4	moderate
≥5.4	poor

Table 1. The original Nottingham Prognostic Index classification.

Antibody	Clone	Source	Pretreatment	Dilution
ER	1D5	DAKO	pH9.0	1:35
PR	636	DAKO	pH9.0	1:50
Her-2/neu	HercepTest	DAKO	pH6.1	RTU
Ki-67	MIB-1	DAKO	pH9.0	1:100
p53	DO-7	DAKO	pH9.0	1:1000

Table 2. Immunohistochemical stains.

Immunohistochemistry

All biopsy samples were previously evaluated by two independent pathologists. The biopsy samples had been fixed in 10% neutral buffered formalin and cut in 3–4 micron thick sections. Antigen was retrieved by incubating the slides in 95 degrees Celzius for 45 minutes in steamer in target retrieval solution, pH6.0 (DAKO, K534011) or pH 9.0, respectively **(Table 2).** The slides were incubated with primary antibody **(Table 2)** for 30 min. The visualization was carried out with dextran polymer conjugated with peroxidase and secondary antibody (EnVision+, DAKO, K534011) for 30 min.

Marker	NPI 1		NPI 2		NPI 3		p-value χ^2 (chi-square) test
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	
ER	1	39	7	33	18	22	p<0.001
	2.50%	97.50%	17.50%	82.50%	45.00%	55.00%	
PR	3	37	10	30	25	15	p<0.001
	7.50%	92.50%	25%	75%	62.50%	37.50%	
Her-2	37	3	32	8	26	14	P<0.001
	92.50%	7.50%	80%	20%	65%	35%	
Ki-67 low	3	37	12	28	31	9	p<0.001
	7.50%	92.50%	30%	70%	77.50%	22.50%	
Ki-67 high	37	3	28	12	9	31	p<0.001
	92.50%	7.50%	70%	30%	22.50%	77.50%	
p53	21	19	19	21	11	29	P=0.057
	52.50%	47.50%	47.50%	52.50%	27.50%	72.50%	

Table 3. Expression of protein biomarkers in different NPI groups.

Interpretation of results

Estrogen and Progesterone Receptors

The stain was interpreted according to the criteria set by Remele and Stegner as immunoreactive score (IS). This score was derived as the percentage of positive cells (scored as 1,2,3 and 4, respectively) with the intensity of staining (scored as 1,2 and 3, respectively). Evaluation was carried out by two independent pathologists. Non-neoplastic epithelium of normal terminal duct-lobular unit was used as an internal control.

Interpretation of Her-2/neu immunoreactivity was carried out according to the international criteria set for the HercepTest interpretation.

Interpretation of proliferative index as measured by Ki-67 was carried out by estimating the percentage of cells with nuclear stain in the most mitoically active areas. The threshold between low and high proliferative rate was 20%.

Interpretation of p53 was carried out by estimating the percentage of positive cells. The threshold for positivity was 10% of the tumor cell nuclei.

Results

Positive expression of ER and PR was inversely related to the NPI numerical value. This difference was statistically significant between the groups (p<0.001). Also, the incidence of ER positive, PR negative cases increased with increasing NPI numerical value. This difference was statistically significant between the groups (p=0.002).

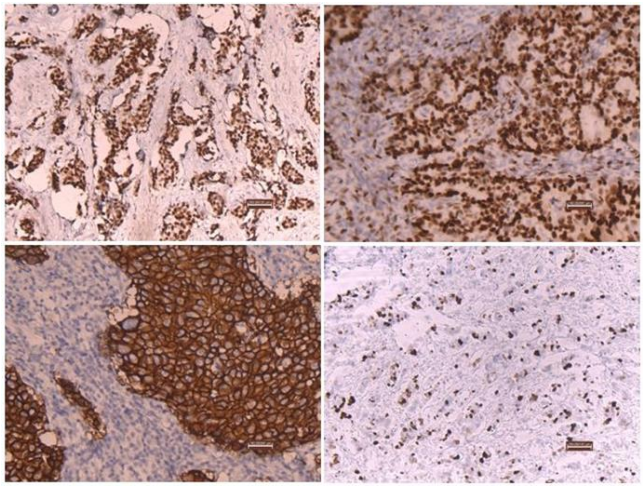

Figure 1. Immunohistochemical stain for Estrogen Receptor (upper-left; immunoperoxidase, 10x), Progesterone Receptor (upper-right; immunoperoxidase, 20x), Her-2/neu (lower-left; immunoperoxidase, 20x) and Ki-67 (lower-right, immunoperoxidase, 10x).

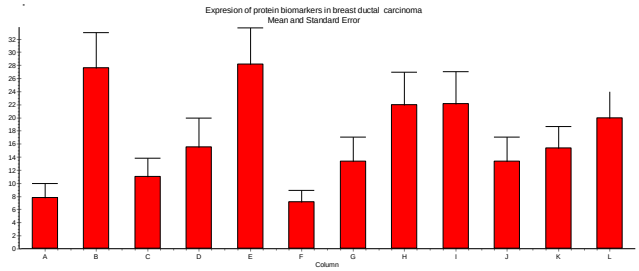

Figure 2. Chi-square test for indepencence: Expression of biomarkers in different NPI groups is significant (p<0.001).

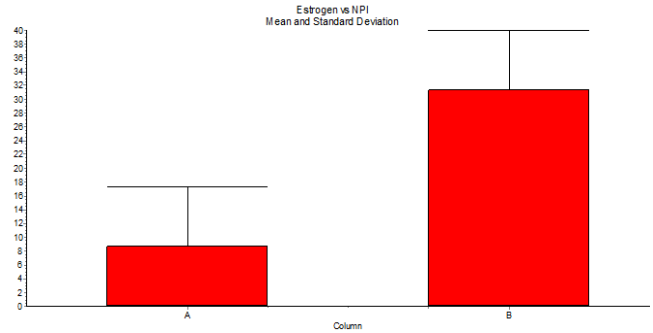

Figure 3. Unpaired t test with Welch correction: Expression of ER in different NPI groups is significant (p<0.001).

Amplification of Her-2/neu was in direct correlation with NPI numerical value. This difference was statistically significant between the groups (p=0.001). Similarly, high rate of Ki-67 increased with increasing NPI value. This difference was statistically significant between the groups (p<0.001). Expression of antioncogenic protein p53 was heterogenously distributed in the NPI groups without any significant difference (p=0.057).

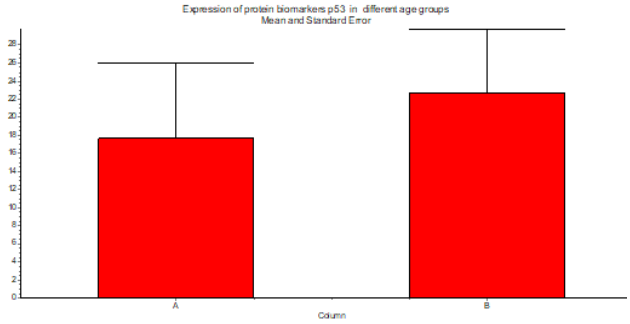

Figure 4. Chi square test for indepencence: Expression of p53 in different age groups shows significant difference (p<0.01).

Marker	pT1		pT2		pT3		pT4		p-value χ^2 (chi-square) test
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	
ER	6	38	18	43	1	6	1	0	p>0.05
	13.64%	86.36%	29.51%	70.49%	14.29%	85.71%	100%	0	
PR	7	37	23	38	3	4	1	0	p>0.05
	15.91%	84.09%	37.71%	62.29%	42.86%	57.14%	100%	0%	
Her-2	40	4	45	16	6	1	0	1	P<0.05
	90.91%	9.09%	73.78%	26.22%	85.72%	14.28%	0%	100%	
Ki-67 low	5	39	30	31	5	2	1	0	P<0.05
	11.37	88.63%	49.19%	50.81%	71.43%	28.57%	100%	0%	
Ki-67 high	39	5	31	30	2	5	0	1	P<0.05
	88.64%	11.36%	50.82%	49.18%	28.58%	71.42%	0%	100%	
P53	23	21	22	39	5	2	0	1	p>0.05
	52.28%	47.72%	36.07%	63.93%	71.43%	28.57%	0%	100%	

Table 4. Expression of protein biomarkers in respective pT stages.

Marker	≤40		41-60		≥61		p-value χ^2 (chi-square) test
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	
ER	3	10	14	49	9	36	p>0.05
	23.08%	76.92%	22.23%	77.77%	20.00%	80.00%	
PR	3	10	20	43	15	30	p>0.05
	23.08%	76.92%	31.75%	68.25%	33.33%	66.67%	
Her-2	9	4	51	12	36	9	p>0.05
	69.24%	30.76%	80.96%	19.94%	80.00%	20.00%	
Ki-67 low	7	6	26	37	14	31	p>0.05
	53.85%	46.15%	41.27%	58.73%	31.11%	68.89%	
Ki-67 high	6	7	37	26	31	14	p>0.05
	46.15%	53.85%	58.73%	41.27%	68.89%	31.11%	
p53	1	12	27	36	25	20	P<0.01
	7.70%	92.30%	42.86%	57.14%	55.56%	44.44%	

Table 5. Expression of protein biomarkers in different age groups.

In contrast, as shown in Table 4, analysis of expression of protein biomarkers in different T

stages showed no significant difference between the groups.

Additionaly, as shown in table 5 and figure 4, analysis of biomarker expression in different age groups showed siginficant difference only for p53.

Discussion

In our study we observed that expression of routinely applied immunohistochemical antibodies namely, ER, PR, Her-2/neu and Ki-67 showed significant differences among the NPI groups when the original, three-tiered NPI system is used (Table 3). Hence, according to these basic observations we decided to use the original, three-tiered NPI classification instead of newer, modified, five-to-six tiered NPI classifications.[9] The threshold of Low versus High Ki-67 proliferation rate was set according to the Sg.Gallen criteria.[9] Additionally, there was a particular observation of PR loss in cases with high numerical NPI value, emphasizing its role as adverse prognostic marker. This was also described in other studies.[13,14]

In a study by the Nottingham group, immunohistochemical analysis of tissue microarrays was carried out for ER, PR, Her-2/neu, EGFR, Her-3, Her-4, p53, CK5/6, CK7/8 and Mucin 1. According to this study, breast carcinoma cases were classified in a similar way as studies employing gene expression profiling.[11,12,16-22]

Benefits of this methodology are lower cost and wider applicability in Institutitions with limited resourses. Luminal A and Luminal B phenotypes are characterized by Her-3, Her-4 positivity in contrast to Luminal N phenotype which is Her-3 and Her-4 negative.[11,12]

Despite inability to prove any significant difference of p53 expression among NPI groups, we observed significant increase of expression of this protein i younger patients. Many data based on molecular and pathological studies support the important role of p53 in breast carcinogenesis.[15] Nevertheless, despite these observations regarding loss of function of p53 in breast carcinoma, mutations of this gene are found in lower frequency compared to other solid tumors.[15,23] During the last years, new knowledge related to the regulation of p53 protein have described new transcription products of p53. These have described alternative molecular mechanisms, besides mutations, through which p53 is deactivated in breast cancer.[15,23] The molecular analysis of different stages of activity of p53 protein may have a diagnostic, prognostic and therapeutic implications in the future.

In our study, we observed significant difference of prevalence of Her-2+/ER- phenotype between three NPI groups whereas Her-2+/ER+ phenotype was not significantly different. This observation also corresponds to the results of the study by Green et al.[3]

Conclusions

The results of our study prove that stratification of patients according to the original, three-tiered, Nottingham Prognostic Index is in correlation with “good” and “bad” prognostic biomarkers. Cases with “favourable immunophenotype” are more prevalent in “favourable NPI groups” whereas the opposite is true for cases with “adverse immunophenotype”. p53 expression is seen more in patients less than fourty years of age, correlating with general view in terms of correlation between young age and adverse prognosis.

Declaration of Interest

Authors declare no competing interests.

Acknowledgements

Nora Shabani designed the study and wrote the manuscript. Fisnik Kurshumliu analyzed data and contributed in writing the manuscript. Ljube Ivkovski analyzed data and contributed in writing the manuscript. Suzana Manxhuka Kërliu provided important contribution in study design and data interpretation.

References

1. Lakhani SR., Ellis IO, Schnitt SJ, Tan PH, van der Vijver MJ. WHO Classification of Tumours of the Breast, Fourth Edition. 2012.
2. Anderson TJ. Breast cancer prognostication in the 21st century and the Nottingham prognostic index. J Clin Pathol. 2002;55(2):86–7.
3. Green AR, Powe DG, Rakha EA, Soria D, Lemetre C, Nolan CC, et al. Identification of key clinical phenotypes of breast cancer using a reduced panel of protein biomarkers. Br J Cancer. 2013;109(7):1886–94.
4. Daniele Soria [a], Jonathan M. Garibaldi [a], Federico Ambrogi [c], Andrew R. Green [b], Des Powe [b], Emad Rakha [b], et al. A methodology to identify consensus classes from clustering algorithms applied to immunohistochemical data from breast cancer patients.Computers in Biology and Medicine. 2010;40:318-330.

5. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res BCR. 2010;12(4):207.
6. Sundquist M, Thorstenson S, Brudin L, Nordenskjöld B. Applying the Nottingham Prognostic Index to a Swedish breast cancer population. South East Swedish Breast Cancer Study Group. Breast Cancer Res Treat. 1999;53(1):1–8.
7. Campbell HE, Taylor MA, Harris AL, Gray AM. An investigation into the performance of the Adjuvant! Online prognostic programme in early breast cancer for a cohort of patients in the United Kingdom. Br J Cancer. 2009;101(7):1074–84.
8. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn H-J, et al. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol Off J Eur Soc Med Oncol ESMO. 2011;22(8):1736–47.
9. Galea MH, Blamey RW, Elston CE, Ellis IO. The Nottingham prognostic index in primary breast cancer. Breast Cancer Res Treat. 1992;22(3):207–19.
10. Anders CA, Johnson R, Litton J, Phillips M, Bleyer A. Breast Cancer Before Age 40 Years. Semin Oncol. 2009;36(3):237-249.
11. Green AR, Powe DG, Rakha EA, Soria D, Lemetre C, Nolan CC, et al. Identification of key clinical phenotypes of breast cancer using a reduced panel of protein biomarkers. Br J Cancer. 2013;109(7):1886–94.
12. Nottingham University Hospitals Research & Innovation Extranet | Nottingham Prognostic Index Plus (NPI+): A Ground Breaking Tool for Breast Cancer http://nuhrise.org/2010/11/nottingham-prognostic-index-plus-npi-a-ground-breaking-tool-for-breast-cancer/
13. Cancello G, Maisonneuve P, Rotmensz N, Viale G, Mastropasqua MG, Pruneri G, et al.Progesterone receptor loss identifies Luminal B breast cancer subgroups at higher risk of relapse. Ann Oncol Off J Eur Soc Med Oncol ESMO. 2013;24(3):661–8.
14. Braun L, Mietzsch F, Seibold P, Schneeweiss A, Schirmacher P, Chang-Claude J, et al. Intrinsic breast cancer subtypes defined by estrogen receptor signalling-prognostic relevance of progesterone receptor loss. Mod Pathol Off J U S Can Acad Pathol Inc. 2013;26(9):1161–71.
15. Yang P, Du CW, Kwan M, Liang SX, Zhang GJ. The impact of p53 in predicting clinical outcome of breast cancer patients with visceral metastasis. Sci Rep. 2013; 3:2246.
16. Naderi A, Teschendorff AE, Barbosa-Morais NL, Pinder SE, Green AR, Powe DG, et al. A gene-expression signature to predict survival in breast cancer across independent data sets. Oncogene. 2007;26(10):1507–16.
17. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;17:406(6797):747–52.
18. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001;11:98(19):10869–74.
19. Sotiriou C, Neo S-Y, McShane LM, Korn EL, Long PM, Jazaeri A, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci. U S A. 2003; 2:100(18):10393–8.
20. Chen C, Dhanda R, Tseng W-Y, Forsyth M, Patt DA. Evaluating use characteristics for the oncotype dx 21-gene recurrence score and concordance with chemotherapy use in early-stage breast cancer. J Oncol Pract Am Soc Clin Oncol. 2013;9(4):182–7.
21. De Boer RH, Baker C, Speakman D, Chao CY, Yoshizawa C, Mann GB. The impact of a genomic assay (Oncotype DX) on adjuvant treatment recommendations in early breast cancer. Med J Aust. 2013; 5:199(3):205–8.
22. DeFrank JT, Salz T, Reeder-Hayes K, Brewer NT. Who gets genomic testing for breast cancer recurrence risk? Public Health Genomics. 2013;16(5):215–22.
23. Purwaningsih NM, Sailan AT, Mohd Sinon SH, Jalil AA. Role of p16 and p53 in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma: A study in Malasia . Journal of International Dental and Medical Research. 2017;10(1):42-47.