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The Role of Serum Nestin in Diagnosis and Prognosis of Breast Cancer: A Prospective Observational Study

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Abstract. *Background/Aim: The molecular classification of breast cancer has enabled targeted therapy for specific molecular subtypes. Nestin, which has been studied for its role in oncogenesis, could contribute to this direction. This study aimed to investigate the differences between serum nestin levels and molecular profiling, as well as histopathological tumor types, in women who underwent surgery for breast cancer. Patients and Methods: Women who underwent surgery for breast cancer at the Breast Unit of the 1st Propaedeutic Department of Surgery, Hippocration General Hospital, National and Kapodistrian University of Athens were prospectively included. Patients' demographic data were recorded and serum nestin levels were measured. Molecular biomarker analysis was performed, as well as histopathologic assessment. Results: Seventy patients were included in the analysis. Among patients with breast cancer, 93% were estrogen receptor (ER) positive, 91% were progesterone receptor (PR) positive, and 43% were human epidermal growth factor receptor 2 (HER2) positive. Ki67 was expressed in 16% of patients and p53 was expressed in 32% of patients. Invasive ductal carcinoma was diagnosed in 80% of patients, with 44% of tumors classified as T1 and 46% as T2. Additionally, 43% were G1 and 56% were N0, while 34% were N1. No statistically significant difference was observed between serum nestin levels and ER, PR, HER2, Ki67, and p53 expression. Furthermore, no difference was observed between serum nestin levels and breast cancer histological type, size, N-stage, and grading. Conclusion: The diagnostic and prognostic role of circulating nestin for breast cancer was not confirmed and no correlation with immunohistochemistry results was observed. Thus, the necessity of larger prospective studies is enhanced.*

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Breast cancer accounts for the most-commonly diagnosed cancer among women and the leading cause of cancer-death worldwide (1). This heterogenous clinical entity derives from the mammary gland's epithelial cells (2). It is classified into four subtypes with distinct clinical value according to different histopathological patterns and biomarker expression, such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and proliferation marker Ki-67 (3). The clinical diversities of these tumors have been better reflected by their gene expression, whereas a new mRNA gene-based subdivision [Luminal A (ER^+/PR^+, $HER2^-$), Luminal B (ER^+/PR^+, $HER2^+$), $HER2^+$ ($ER^-/PR^-/HER2^+$) and basal-like/triple-negative ($ER^-/PR^-/HER2^-$)] seems to predict prognosis more accurately (4). This molecular classification has also enabled classification according to gene expression profiling, which could propose targeted-therapy for specific molecular subtypes, like triple-negative tumors (5, 6).

The high cost of gene expression panels has prevented their wide application in every day clinical practice, arising the need for precise, low-cost, and highly accessible biomarkers that could accurately distinguish breast cancer

molecular subtypes and potentially provide precise therapeutic choices (7, 8). Nestin is a type VI intermediate filament originally expressed in neural stem cells that has been studied for its role in oncogenesis including cell migration, cell proliferation, cell adhesion and neo-angiogenesis (9). Research related to solid tumors revealed poor prognosis and higher recurrence rates in "nestin-rich" patients with breast, ovarian, esophageal, hepatocellular carcinoma, gastric and gallbladder carcinomas (10, 11). However, limited data derive from studies with serum nestin evaluation. Sal *et al*. demonstrated significantly higher serum nestin levels in patients with ovarian malignancy in comparison with those with ovarian benign tumors (median: 12.4±8.98 ng/ml *vs*. 6.9±2.97 ng/ml, p=0.001), whereas non statistically significant difference was observed among the four pathological subtypes (12).

The aim of this study was to investigate the differences between soluble nestin levels in blood samples from patients with breast cancer and healthy controls. Correlations between blood serum nestin and tumor characteristics, such as histological type, tumor size, stage of the disease according to the TNM criteria (WHO), hormone and c-erb2 tumor receptors, ki67 index and p53 molecule were evaluated attempting to propose a useful biomarker that could indicate each of the four subtypes.

Patients and Methods

Serum samples were prospectively obtained from 70 patients who underwent breast cancer surgery in the Breast Unit of the First Propaedeutic Department of Surgery, "Hippocration" Athens General Hospital from March 2020 to March 2021. Exclusion criteria were suspicious secondary malignancies, systemic disease, renal and/or hepatic impairment, congestive heart failure or chronic respiratory disease, neoadjuvant chemotherapy, history of chemotherapy or radiotherapy for any malignancy and pregnancy. Histopathologic assessment was performed by two experienced pathologists. Data on histological type, tumor size, stage of the disease according to the TNM criteria (WHO), hormone and C-ERB2 tumor receptors, ki67 index and p53 were retrieved from all breast cancer cases. No pre-operative serum nestin values were available. This study was approved by the Ethical Committee of "Hippocration" Athens General Hospital and was conducted in compliance with the Declaration of Helsinki guidelines. A written informed consent was obtained by all patients before participation in the study.

Biochemical analysis. Blood samples were collected in EDTA-containing tubes and anticoagulant-free tubes after an overnight fast. Plasma and serum were separated immediately and stored at –80˚C until analysis.

Measurement of serum nestin levels. Serum nestin levels were measured using a solid-phase, enzyme-linked immunosorbent assay (ELISA) kit (YH-Biosearch, Shanghai, PR China, Cat.No: YHB3353Hu), based on the sandwich principle, according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was performed using SPSS (International Business Machines Corporation – IBM, Armonk, NY, USA) version 16.0. Univariable analysis of the relationship between serum nestin levels and molecular biomarkers was performed using the non-parametric Mann-Whitney *U*-test. Univariable analysis of the relationship between serum nestin levels and histopathological features of breast tumors was performed using the non-parametric Kruskal-Wallis test. All reported confidence interval (CI) values were calculated at the 95% level. A p-value <0.05 was defined as the level of statistical significance.

Results

In total, 70 patients were included in the analysis. Among patients with breast cancer, 93% were ER positive, 91% were PR positive, and 43% were HER2 positive. Ki67 was expressed in 16% of patients and p53 was expressed in 32% of patients. Histopathological subtypes were classified into four categories as follows: 80% IDC, 16% invasive lumbar carcinoma (ILC), 3% ductal carcinoma in situ (DCIS) and 1% mixed type (IDC-ILC). In terms of histopathological staging 44% of tumors were T1, 46% were T2, and 10% were T3. Tumor grading revealed 43% G1 tumors, 37% G2 tumors, and 20% G3 tumors. Finally, lymph node invasion was reported as follows: 56% N0, 34% N1, 6% N2, and 4% N3 (Table I).

Molecular biomarkers. Mean serum nestin level in ER positive patients was 107.5±42.5 pg/ml and 99.6±34.6 pg/ml in ER negative patients (p=0.734). In addition, tumor PR expression was not significantly correlated with serum nestin levels (mean serum nestin 107.7±42.8 pg/ml in PR positive tumors *vs*. 98.7±31 pg/ml in PR negative tumors, p=0.613). Mean serum nestin was 98.6±41.8 pg/ml in HER2 positive tumors and 112.5±41.3 in HER2 negative tumors (p=0.552) (Table II). Moreover, no relationship was indicated between serum nestin levels and tumor Ki67 expression (p>0.05), as well as tumor p53 expression (p>0.05).

Histopathological analysis. Univariable analysis demonstrated no statistically significant correlations between serum nestin levels histopathological types of patients with breast cancer as the mean serum nestin levels were 109.5±39 pg/ml in patients with IDC, 106.8±54.3 pg/ml in patients with ILC and 62±33.9 pg/ml in patients with DCIS (p=0.178). In addition, no significant differences were observed in serum nestin levels of patients with different tumor sizes (mean serum nestin levels of 99.4±45.1 pg/ml in patients with T1 tumors, 119.8±35.3 pg/ml in T2 tumors and 83.3±38.4 pg/ml in T3 tumors, p=0.068). Mean serum nestin levels were 107±44.7 pg/ml in N0 tumors, 106.7±43 pg/ml in N1 tumors, 110±22.2 pg/ml in N2 tumors and 103.3±8.3 pg/ml in N3 tumors (p=0.998). Finally, no statistically significant differences were observed in serum nestin levels of patients

Table I. *Patient characteristics regarding tumor biomarker expression and histopathological features.*

	No of patients	Percentage
ER expression		
+	65	93%
–	5	7%
PR expression		
+	64	91%
–	6	9%
HER2 expression		
+	30	43%
–	40	57%
Ki67 expression		
+	11	16%
–	59	84%
p53 expression		
+	22	32%
–	48	68%
Histological type		
IDC	56	80%
ILC	11	16%
DCIS	2	3%
IDC/ILC	1	1%
Size		
T1	31	44%
T2	32	46%
T3	7	10%
N-stage		
N0	39	56%
N1	24	34%
N2	4	6%
N3	3	4%
Grade		
G1	30	43%
G2	26	37%
G3	14	20%

ER: Estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; DCIS: ductal carcinoma *in situ*.

Table II. *Univariable analysis of serum nestin levels among breast cancer patients with different ER, PR, and HER2 expression.*

	Serum nestin, mean±SD (pg/ml)	*p*-Value
ER expression		
+	107.5±42.5	0.734
–	99.6±34.6	
PR expression		
+	107.7±42.8	0.613
–	98.7±31	
HER2 expression		
+	98.6±41.8	0.552
–	112.5±41.3	

ER: Estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2.

Table III. *Univariable analysis of serum nestin levels among breast cancer patients with tumors of different histological types, size, N-status, and histopathological grades.*

	Serum nestin, mean±SD (pg/ml)	*p*-Value
Histological type		
IDC	109.5±39	0.178
ILC	106.8±54.3	
DCIS	62±33.9	
Size		
T1	99.4±45.1	0.068
T2	119.8±35.3	
T3	83.3±38.4	
N-stage		
N0	107±44.7	0.998
N1	106.7±43	
N2	110±22.2	
N3	103.3±8.3	
Grade		
G1	105.4±40.8	0.859
G2	110.5±49.6	
G3	103.2±27.8	

IDC: Invasive ductal carcinoma; ILC: invasive lobular carcinoma; DCIS: ductal carcinoma *in situ*.

with breast cancer with different grade tumors (mean serum nestin levels 105.4±40.8 pg/ml in G1 tumors, 110.5±49.6 pg/ml in G2 tumors and 103.2±27.8 pg/ml in G3 tumors, p=0.859) (Table III).

Discussion

The need for early diagnosis and accurate prognosis of cancer, as well as evolutions in molecular biology, have led investigators to develop new sensitive and specific assays for timely and reliable malignancy detection (13). Nestin has been proposed as a potential independent prognostic factor for breast cancer, while studies seem to provide conflicting results. A meta-analysis of 15 studies by Zhang *et al*. showed that positive nestin expression was associated with reduced breast cancer specific survival (HR=1.30, 95%CI=1.06-1.60, p=0.01) and overall survival (HR=1.89, 95%CI=1.34-2.67, p=0.0003) on multivariate analysis. Also, a positive correlation was indicated with higher grade, larger tumor size, positive blood vessel invasion, high vascular proliferation index and expression of basal-like markers cytokeratin 5 (CK5), P-cadherin, and epidermal growth factor receptor (EGFR) (14).

The first study regarding nestin blood serum levels was performed by Aglan *et al*. 2021 who collected blood serum

from 49 patients with invasive breast cancer (15). Nestin was found at lower levels in patients with larger (>5 cm) tumor sizes (27 *vs*. 31.6 pg/ml), but no statistical significance was reached. Lymph node, distal metastasis, tumor staging, and molecular intrinsic subtypes were not associated with serum nestin levels. Our study showed no statistically significant correlations between serum nestin levels of breast cancer patients and ER, PR, HER-2, as well as tumor histological subtypes. In addition, no correlation between serum nestin levels and tumor ki67 or p53 expression was found, both indicators of aggressive behavior of tumors. No significant difference of serum nestin levels was observed in breast cancer patients according to their N-status and their tumor grade. Similarly, in the study by Aglan *et al*. 2021, there was a tendency for higher levels of nestin in patients with higher tumor grade (37.7 *vs*. 31.5 pg/ml), without reaching statistical significance.

Krüger *et al*. investigated nestin expression using immunohistochemistry in tissues of patients with breast cancer (16). They observed nestin positivity in only 9-28% of studied patients with breast cancer. However, they assumed that increased nestin expression can be only limited to cases of basal intrinsic subtype that are highly undifferentiated and associated with Cytokeratin, P-cadherin, and EGFR staining. In fact, nestin protein expression, assayed using immunohistochemistry, was consistently associated with higher histological grade (OR=2.7-14.3), larger tumours (OR=1.8-2.4), lymph node negativity (OR=3.3) and more frequent *BRCA1* mutations (OR=8.7, $p<0.0005$). Nestin expression was also found to be associated with higher risk of basal-like differentiation as indicated by the increased expression of cytokeratin 5 (OR=8.7-13.8, $p<0.0005$), EGFR (OR=3.7-8.2, $p\leq0.05$) and P-cadherin (OR=7.0-8.9, $p<0.0005$). Nestin protein expression was associated with reduced breast cancer specific survival ($p=0.002$).

More recently, Hewala *et al*. studied the sensitivity and specificity of serum nestin in patients who underwent surgery and received chemotherapy for breast cancer (17). In the breast cancer group, the median serum nestin levels were 10.50 (range=8.32-15.06 ng/ml) before surgery, 8.87 (range=2.01-12.69 ng/ml) after surgery and 8.72 (range=7.56-11.06 ng/ml,) after chemotherapy ($p=0.045$). Statistical analysis revealed that the serum levels of nestin were significantly higher in the breast cancer group compared to the control group ($p<0.001$). Receiver operating characteristic (ROC) curve analysis revealed a sensitivity of 80% and specificity of 75% for serum nestin levels, as well as a cut-off point of 9.90 ng/ml. The association of nestin with non-favorable oncological outcomes has been indicated in patients with other malignancies as well. For instance, the overall survival was shorter for the nestin-positive patients with high-grade epithelial ovarian cancer who underwent cytoreductive surgery after neoadjuvant therapy compared to nestin-negative patients (48 *vs*. 67 months, respectively), even though this difference did not reach statistical significance ($p=0.43$) (18).

The current study is the third to evaluate nestin levels in blood samples of patients with breast cancer in correlation to the tumor histopathological characteristics, grading, staging, hormonal receptor expression and lymph node involvement. Study findings are in concordance with the other similar studies available in the literature. However, some limitations should be mentioned. First, no nestin expression was evaluated using immunohistochemistry; thus, no correlation at the level of proteomics could be assessed. In addition, lack of healthy participants underpowers our study protocol. Further studies with prospectively recruited patients and healthy controls should be initiated to further examine the role of nestin.

Conclusion

Emerging evidence highlights nestin as an important factor for breast cancer progression. Although most studies have shown statistically significant correlation between serum nestin expression and breast tumours, immunohistochemistry seems to be the gold standard method. In our study, nestin levels in circulating blood were evaluated as a diagnostic biomarker for breast cancer, but also as a surrogate marker for tumors' worrisome clinical and histopathological characteristics. Nevertheless, no similar results were illustrated in immunohistochemistry. Therefore, more prospective studies including multivariable analysis should be conducted in order to determine the role of nestin in diagnosis and prognosis of breast cancer.

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None to disclose.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Aikaterini Kolia: Conceptualization, writing – Original draft, data curation; Maximos Frountzas: Writing – review and editing; Efstathia Liatsou: Writing – Original draft; George Samelis: Funding acquisition; Flora Zagouri: Methodology; George C. Zografos: Supervision; Maria Gazouli: Validation; Nikolaos V. Michalopoulos: Project administration.

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References

1 Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, Jemal A, Siegel RL: Breast Cancer Statistics, 2022. CA Cancer J Clin 72(6): 524-541, 2022. DOI: 10.3322/caac.21754

2 Lamb CA, Vanzulli SI, Lanari C: Hormone receptors in breast cancer: more than estrogen receptors. Medicina (B Aires) 79(Spec 6/1): 540-545, 2019.

3 Malhotra GK, Zhao X, Band H, Band V: Histological, molecular and functional subtypes of breast cancers. Cancer Biol Ther 10(10): 955-960, 2010. DOI: 10.4161/cbt.10.10.13879

4 Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D: Molecular portraits of human breast tumours. Nature 406(6797): 747-752, 2000. DOI: 10.1038/35021093

5 Chan HL, Fung S, Seto WK, Chuang WL, Chen CY, Kim HJ, Hui AJ, Janssen HL, Chowdhury A, Tsang TY, Mehta R, Gane E, Flaherty JF, Massetto B, Gaggar A, Kitrinos KM, Lin L, Subramanian GM, McHutchison JG, Lim YS, Acharya SK, Agarwal K, GS-US-320-0110 Investigators: Tenofovir alafenamide *versus* tenofovir disoproxil fumarate for the treatment of HBeAg-positive chronic hepatitis B virus infection: a randomised, double-blind, phase 3, non-inferiority trial. Lancet Gastroenterol Hepatol 1(3): 185-195, 2016. DOI: 10.1016/S2468-1253(16)30024-3

6 Verhelst D, Monge M, Meynard JL, Fouqueray B, Mougenot B, Girard PM, Ronco P, Rossert J: Fanconi syndrome and renal failure induced by tenofovir: A first case report. Am J Kidney Dis 40(6): 1331-1333, 2002. DOI: 10.1053/ajkd.2002.36924

7 Laterza OF, Hendrickson RC, Wagner JA: Molecular biomarkers. Drug Information J 41(5): 573-585, 2007. DOI: 10.1177/009286150704100504

8 Seale KN, Tkaczuk KHR: Circulating biomarkers in breast cancer. Clinical Breast Cancer 22(3): e319-e331, 2022. DOI: 10.1016/j.clbc.2021.09.006

9 Nowogrodzka K, Jankowska-Konsur A: Emerging biomarker in carcinogenesis. Focus on Nestin. Postepy Dermatol Alergol 39(6): 1001-1007, 2022. DOI: 10.5114/ada.2022.122599

10 Szymańska-Chabowska A, Świątkowski F, Jankowska-Polańska B, Mazur G, Chabowski M: Nestin expression as a diagnostic and prognostic marker in colorectal cancer and other tumors. Clin Med Insights Oncol 15: 11795549211038256, 2021. DOI: 10.1177/11795549211038256

11 Hwang HS, Park CW, Song MJ: Tenofovir-associated Fanconi syndrome and nephrotic syndrome in a patient with chronic hepatitis B monoinfection. Hepatology 62(4): 1318-1320, 2015. DOI: 10.1002/hep.27730

12 Sal V, Kahramanoglu I, Bese T, Demirkiran F, Sofiyeva N, Soyman Z, Durmus S, Gelisgen R, Arvas M, Uzun H: Is serum level of nestin useful in detecting epithelial ovarian cancer? J Obstet Gynaecol Res 43(2): 371-377, 2017. DOI: 10.1111/jog.13220

13 Beenken SW, Grizzle WE, Crowe DR, Conner MG, Weiss HL, Sellers MT, Krontiras H, Urist MM, Bland KI: Molecular biomarkers for breast cancer prognosis: coexpression of c-erbB-2 and p53. Ann Surg 233(5): 630-638, 2001. DOI: 10.1097/00000658-200105000-00006

14 Zhang X, Xing C, Guan W, Chen L, Guo K, Yu A, Xie K: Clinicopathological and prognostic significance of nestin expression in patients with breast cancer: a systematic review and meta-analysis. Cancer Cell Int 20: 169, 2020. DOI: 10.1186/s12935-020-01252-5

15 Aglan SA, Elsammak M, Elsammak O, El-Bakoury EA, Elsheredy HG, Ahmed YS, Sultan MH, Awad AM: Evaluation of serum Nestin and HOTAIR rs12826786 C>T polymorphism as screening tools for breast cancer in Egyptian women. J Med Biochem 40(1): 17-25, 2021. DOI: 10.5937/jomb0-25295

16 Krüger K, Wik E, Knutsvik G, Nalwoga H, Klingen TA, Arnes JB, Chen Y, Mannelqvist M, Dimitrakopoulou K, Stefansson IM, Birkeland E, Aas T, Tobin NP, Jonassen I, Bergh J, Foulkes WD, Akslen LA: Expression of Nestin associates with BRCA1 mutations, a basal-like phenotype and aggressive breast cancer. Sci Rep 7(1): 1089, 2017. DOI: 10.1038/s41598-017-00862-w

17 Hewala TI, Kamel MS, Elwany YN, Zekry NZA: The value of serum nestin in monitoring the effects of surgery and chemotherapy in female breast cancer patients: a comparison with serum CA15.3. Middle East J Cancer 15(2): 136-144, 2024. DOI: 10.30476/mejc.2023.98136.1886

18 Veyssière H, Aldarazi G, Molnar I, Durando X, Radosevic-Robin N: Nestin as a prognostic biomarker in high-grade epithelial ovarian cancer treated by neoadjuvant chemotherapy. Anticancer Res 42(7): 3583-3594, 2022. DOI: 10.21873/anticanres.15845

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