Open Access Article

Ann. Acad. Med. Siles. (online) 2022; 76: 96–105
eISSN 1734-025X
DOI: 10.18794/aams/149208
www.annales.sum.edu.pl

PRACA ORYGINALNA
ORIGINAL PAPER

Serum levels of VEGF-A, sVEGFR-2 and galectin-3 do not correlate with clinical stage, tumor size, or effectiveness of perioperative chemotherapy in patients with non-metastatic breast cancer

Stężenie VEGF-A, sVEGFR-2 i galektyny-3 w surowicy pacjentek z rakiem piersi nie koreluje ze stopniem zaawansowania klinicznego, wielkością guza ani skutecznością chemioterapii okołooperacyjnej

Iga Grochoła-Małecka[1], Paulina Czajka-Francuz[1], Aleksander J. Owczarek[2], Jerzy Wojnar[1], Gabriela Handzlik[1], Tomasz Francuz[3], Jerzy Chudek[1]

[1]Klinika Chorób Wewnętrznych i Chemioterapii Onkologicznej, Samodzielny Publiczny Szpital Kliniczny im. A. Mielęckiego, Śląski Uniwersytet Medyczny w Katowicach
[2]Zakład Promocji Zdrowia i Leczenia Otyłości, Wydział Nauk Medycznych w Katowicach, Śląski Uniwersytet Medyczny w Katowicach
[3]Katedra i Zakład Biochemii, Wydział Nauk Medycznych w Katowicach, Śląski Uniwersytet Medyczny w Katowicach

ABSTRACT

INTRODUCTION: Tumor angiogenesis is regulated by numerous cytokines and growth factors, with vascular endothelial growth factor (VEGF), soluble vascular endothelial growth factor receptor 2 (sVEGFR-2), and galectin-3, playing a significant role in the process. There are conflicting data concerning changes in serum VEGF, sVEGFR-2 and galectin-3 levels in breast cancer (BC) patients during the course of the disease and chemotherapy (CTH). This study aimed to assess the serum levels of VEGF-A, sVEGFR-2, and galectin-3 in women starting adjuvant and neoadjuvant therapy for BC, and their changes during the treatment.

MATERIAL AND METHODS: This single-center study enrolled 98 women with non-metastatic BC, including 56 who started adjuvant therapy and 42 preoperative (neoadjuvant/induction) CTH. The serum levels of VEGF-A, sVEGFR-2, and galectin-3 were assessed at the beginning of CTH and after 2 subsequent months.

RESULTS: There were no significant differences in the serum levels of VEGF-A, sVEGFR-2, and galectin-3 between patients starting adjuvant and preoperative therapy. In addition, there was no correlation between the serum levels and the clinical stage of BC. During CTH, a significant increase in VEGF-A, sVEGFR-2, and galectin-3 was noted, however, without a predictive significance for obtaining complete pathological response (pCR) both for the initial levels and changes in the serum levels.

Received: 02.02.2022 **Revised:** 13.04.2022 **Accepted:** 13.04.2022 **Published online:** 29.09.2022

Address for correspondence: lek. Iga Grochoła-Małecka, Klinika Chorób Wewnętrznych i Chemioterapii Onkologicznej, Samodzielny Publiczny Szpital Kliniczny im. A. Mielęckiego, Śląski Uniwersytet Medyczny w Katowicach, ul. Reymonta 8, 40-027 Katowice, tel. +48 32 259 12 17, e-mail: groc.iga@gmail.com

Publisher: Medical University of Silesia, Katowice, Poland

CONCLUSIONS: The serum levels of VEGF-A, sVEGFR-2, and galectin-3 do not correlate with the clinical stage or tumor size in patients with non-metastatic BC. The baseline levels of VEGF-A, sVEGFR-2 and galectin-3, and the observed increase in the serum levels of VEGF-A and sVEGFR-2 during CTH do not predict its efficacy.

KEY WORDS

breast cancer, angiogenesis, galectin-3, vascular endothelial growth factor, chemotherapy

STRESZCZENIE

WSTĘP: Angiogeneza nowotworowa jest procesem regulowanym przez wiele cytokin i czynników wzrostu, spośród których znaczącą rolę odgrywają czynnik wzrostu śródbłonka naczyń (*vascular endothelial growth factor* – VEGF), drugi rozpuszczalny receptor dla śródbłonkowego czynnika wzrostu (*soluble vascular endothelial growth factor receptor 2* – sVEGFR-2) i galektyna-3. Dane literaturowe dotyczące oceny zmian stężenia VEGF, sVEGFR-2 oraz galektyny-3 w trakcie chemioterapii (*chemotherapy* – CTH) raka piersi (*breast cancer* – BC) są niejednoznaczne. Celem niniejszej pracy była analiza stężenia VEGF-A, sVEGFR-2 oraz galektyny-3 w surowicy pacjentek z rakiem piersi, rozpoczynających adjuwantową i neoadjuwantową chemioterapię oraz ocena zmian stężenia tych cytokin w trakcie leczenia.
MATERIAŁ I METODY: Jednoośrodkowe badanie objęło 98 pacjentek z miejscowo zaawansowanym rakiem piersi, w tym 56 poddanych adjuwantowej i 42 neoadjuwantowej terapii. Stężenie VEGF-A, sVEGFR-2 i galektyny-3 w surowicy krwi oceniono na początku leczenia oraz po 2 miesiącach terapii.
WYNIKI: Nie stwierdzono istotnych różnic pomiędzy stężeniami VEGF-A, sVEGFR-2 oraz galektyny-3 w surowicy pacjentek poddanych adjuwantowej i neoadjuwantowej chemioterapii. Nie wykazano również zależności między stężeniem tych cytokin w surowicy a stopniem zaawansowania klinicznego raka piersi. W trakcie przedoperacyjnej chemioterapii odnotowano znaczące zwiększenie stężenia VEGF-A, sVEGFR-2 i galektyny-3, jednakże zarówno wyjściowe stężenia cytokin, jak i zmiany w czasie nie miały znaczenia predykcyjnego dla uzyskania całkowitej odpowiedzi patologicznej.
WNIOSKI: Stężenia VEGF-A, sVEGFR-2 oraz galektyny-3 w surowicy nie korelują ze stopniem zaawansowania klinicznego ani masą nowotworu u pacjentek z miejscowo zaawansowanym rakiem piersi. Wyjściowe stężenia VEGF-A, sVEGFR-2 i galektyny-3 oraz zaobserwowany wzrost stężeń tych cytokin w surowicy w trakcie chemioterapii nie mają wartości predykcyjnej dla jej skuteczności.

SŁOWA KLUCZOWE

rak piersi, angiogeneza, galektyna-3, czynnik wzrostu śródbłonka naczyń, chemioterapia

INTRODUCTION

Breast cancer (BC) is the most common malignancy in women in developed countries and a growing health problem in developing countries. In 2020, in the European Union, over 530,000 women were diagnosed with BC, about 140,000 died, and 2 million live with cancer diagnosed in the last 5 years [1].

Tumor angiogenesis is a process of blood vessel development from pre-existing vasculature, necessary for oxygen and nutrient supplies during tumor growth. This process is implicated in the progression and metastasis of BC, like other solid tumors. High microvessel densities (MVD) reflecting intensive tumor angiogenesis, predicts poor outcomes: disease--free survival (DFS) and overall survival in invasive BC [2].

Angiogenesis is regulated by many cytokines, growth factors, adhesive molecules, and enzymes, among which the vascular endothelial growth factor (VEGF) appears to play a key role in the stimulation of endothelial cell migration. An increased expression of VEGF family cytokines has been reported among others in breast, colorectal, prostate, kidney, and bladder cancers [3].

The VEGF family consists of several subtypes, among which the most important are VEGF-A, VEGF-B, and VEGF-C. VEGF-A and VEGF-B exert biological effects by means of specific receptors with tyrosine kinase activity – VEGFR-1 and VEGFR-2 [4].

VEGF-A plays a significant role in inducing endothelial cell proliferation, migration, proteolytic activity, stimulating microvascular leakage, and promoting angiogenesis. VEGF-A can stimulate lymphangiogenesis indirectly, recruiting bone marrow--derived macrophages, which release lymphangiogenic growth factors VEGF-C and VEGF-D. Thus, VEGF-A increases both pathological hemangiogenesis and lymphangiogenesis [5]. Moreover, VEGF-A increases matrix metalloproteinase activity, and shows chemotactic action for macrophages and granulocytes [6].

VEGFR-2 signaling dominates in the transduction of proliferative effects of VEGF-A [7]. Both receptors are found, among others, on vascular endothelial cells and

cancer cells, including BC cells, and stimulate cell migration [8]. In contrast, normal mammocytes do not express these receptors. The overexpression of VEGFR-1 and VEGFR-2 in BC cells was associated with histological markers of aggressiveness [9]. In addition, tumor stromal VEGF-A expression was associated with unfavorable clinical outcomes – shorter cancer-specific survival (CSS) and DFS in inflammatory BC [10].

Besides VEGF receptors bound to the cell membrane, these receptors also exist in a soluble form (sVEGFR-1 and sVEGFR-2) generated by alternative splicing, with a potential function of decoy receptors [11,12]. sVEGFR-2 has a slightly lower affinity for binding to VEGF-A than sVEGFR-1 [13]. Binding of the members of the VEGF family may reduce the availability of these cytokines for membrane receptors, thereby negatively regulating VEGFR-mediated signaling [14]. The intratumoral presence of sVEGFR--1 was confirmed in breast tumor tissues, but with no correlation with clinicopathological factors [15]. Increased serum concentrations of sVEGFR-1 and sVEGFR-2, proportional to the clinical stages were shown in a small cohort of women with BC [16]. The prognostic significance of both sVEGFR for the clinical outcomes has not yet been researched.

The group of mediators regulating tumor angiogenesis includes galectin-3 [17]. This lectin has two domains (N-terminal and C-terminal) responsible for its activity in the extracellular space, among others enhanced tumour cell adhesion to the extracellular matrix and increased metastatic spreading, the inhibition of apoptosis, stimulation of cell proliferation, and promotion of angiogenesis [17,18]. Galectin-3 expression in cancer cells can be shown in the cytoplasm, cell nucleus, and close to the cell membrane. Of note, the biological activity of galectin--3 depends on cellular localization [19]. Its decreased tissue expression was observed in tumors with more pronounced angiogenesis, which correlated with shorter progression-free survival [20]. In BC, only part of the researchers confirmed a correlation between decreased galectin-3 expression and neoangiogenesis [20,21].

The increased serum concentration of this lectin was observed not only in patients with ovarian, rectal, lung, head, and neck cancers but also in patients with BC [21,22].

There are conflicting data concerning the association between serum galectin-3 levels and clinical outcomes and response to chemotherapy (CTH) in BC patients. An increased expression of galectin-3 in the tumor stroma, but not plasma levels, in response to neoadjuvant CTH, was associated with DFS [23].

This study aimed to assess the serum levels of VEGF-A, sVEGFR-2, and galectin-3 in women starting adjuvant and neoadjuvant therapy for BC, and their changes during the treatment.

MATERIAL AND METHODS

This single-center study enrolled 100 women with non-metastatic BC treated in the Department of Internal Diseases and Oncological Chemotherapy from July 2014 to September 2019. The inclusion criteria for the study group were as follow: 1) histologically confirmed BC, 2) clinical stages I-III according to the 8th edition of the American Joint Committee on Cancer (AJCC), 3) before surgery (neoadjuvant/induction subgroup) or after mastectomy/breast-conserving therapy (BCT). The exclusion criteria were as follow: 1) the development of a second malignant tumor in another organ than the breast or axillary lymph nodes during the observation (n = 0), 2) pregnancy and breastfeeding (n = 0), 3) history of autoimmune disease (n = 2). Finally, the analysis included 98 BC patients: 56 patients starting adjuvant therapy (ADJ subgroup) and 42 patients starting preoperative (neoadjuvant/induction) CTH (NEO-ADJ subgroup).

Surveillance after CTH was performed according to the recommendations of the National Comprehensive Cancer Network (NCCN). The patients underwent a physical examination 2–4 times per year for 5 years, then annually. Imaging studies for metastases screening were carried out in symptomatic patients.

The study was approved by the Bioethics Committee of the Medical University of Silesia, Katowice, Poland (KNW/0022/KB1/62/15). Informed consent was obtained from each patient.

Laboratory measurements

Peripheral blood samples (5 ml) were obtained from the patients two times: at the beginning of CTH and after two subsequent months. After centrifugation at 3000 rpm for 10 minutes, the serum samples were transferred to tubes that were stored frozen in liquid nitrogen until analysis. The serum concentrations of VEGF-A, sVEGFR-2 (Thermo Fisher Scientific, MA, U.S.) and galectin-3 (R&D Systems, Inc. Minneapolis, MN, U.S.) were measured using the multiplex technique Bio-Plex (Bio-Rad®, CA, U.S.) according to the manufacturer's manual. Bead fluorescence readings were taken by means of the Bio-Plex 200 System and analyzed with the Bio-Plex Manager version 6.1.0.727 (Bio-Rad®, CA, U.S.).

Data analysis

The postoperative pathological assessments of the surgical specimens were made according to the pathological TNM system [24,25,26]. The assessment included:

– the number, location, and maximum diameter of the removed tumors

– the total number of excised and positive lymph nodes as well as the extent of metastases in the lymph nodes (i.e. micrometastases, macrometastases)
– the histological type and grade of the tumors
– evaluation of the resection margins, including the location and minimum distance of the margin
– vascular invasion and biomarker analysis, including ER, PgR, HER2, and Ki67 status.

The pathological response to preoperative treatment was analyzed in accordance with Pinder classification [27]. Shortly afterwards, a complete pathological response (pCR) was reported if no residual cancer tissue or only cancer in situ was found after surgery in the breast tissue and axilla. A partial response (pPR) was recorded, when a minimal residual disease was found (less than 10% of invasive tumor left) or there was a or 10–50% invasive tumor left or more than 50% invasive cancer left tissue with the post-CTH effect. No response (NR) was reported if there were no signs of response in the breast tissue.

Statistical analysis

Statistical analyses were performed using STATISTICA 13.3 PL (TIBCO Software Inc., Palo Alto, CA, USA) and R software (R Core Team (2013), R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/). The statistical significance was set at a p-value below 0.05. All the tests were two-tailed. Imputations were not performed for missing data. Nominal and ordinal data were expressed as percentages. Interval data were expressed as the mean value ± standard deviation in the case of normal distribution. In the case of data with skewed or non-normal distribution, they were expressed as the median, with lower and upper quartiles. The distribution of variables was evaluated by the Anderson-Darling test and the quantile-quantile (Q–Q) plot. The homogeneity of variances was assessed by the Levene test. Nominal and ordinal data were compared with the χ^2 test. Comparisons between the groups for interval data, including longitudinal data, were performed with ANOVA analysis with contrasts as a post-hoc test (with either raw variables or after logarithmic transformation in the case of non-normal data distribution).

RESULTS

The study group consisted of 56 women starting adjuvant CTH and 42 women starting neoadjuvant/induction therapy for stages I-III BC (Table I). The patients in the ADJ subgroup were significantly older than the women starting preoperative treatment (59 ± 10 and 54 ± 12, $p < 0.05$). The subgroups differed in tumor size and stage, but not in lymph node involvement or pathology grading. In both subgroups, the largest part of patients had hormone-receptor--positive/HER2 negative (HR+/HER2-) BC. Among comorbidities, hypertension was diagnosed in more than half of the patients.

Adjuvant therapy was based on doxorubicin with cyclophosphamide with or without taxane (N = 26), doxorubicin with a taxane (N = 2), taxane with trastuzumab (N = 10), 5-fluorouracil with epirubicin and cyclophosphamide (N = 1), or hormonal therapy only (N = 2).

Table I. Characteristics of patients treated with adjuvant therapy (ADJ subgroup) and preoperative chemotherapy (NEO-ADJ subgroup)
Tabela I. Charakterystyka pacjentek leczonych chemioterapią adjuwantową (podgrupa ADJ) oraz neoadjuwantową (podgrupa NEO-ADJ)

Parameters	ADJ subgroup N = 56 (57.1%)	NEO-ADJ subgroup N = 42 (42.8%)	P
1	2	3	4
Age, years	59 ± 10	54 ± 12	< 0.05
Age, years, n (%)			
< 60	26 (46.4)	27 (64.3)	0.08
≥ 60	30 (53.6)	15 (35.7)	
Clinical stage, n (%)			
I	14 (25.0)	2 (4.8)	< 0.01
II	33 (58.8)	25 (59.5)	
III	9 (9.2)	15 (35.7)	
Tumor size, n (%)			
T1	21 (37.5)	3 (7.2)	
T2	32 (57.1)	25 (59.5)	< 0.001
T3	1 (1.8)	10 (23.8)	
T4	2 (3.6)	4 (9.5)	

cd. tab. I

1	2	3	4
Lymph node involvement, n (%)			
N0	33 (59.9)	16 (38.1)	
N1	17 (30.4)	18 (42.9)	0.12
N2	5 (8.9)	8 (19.0)	
N3	1 (1.8)	0	
Grade, n (%)			
1	6 (10.9)	2 (4.8)	0.53
2	38 (69.1)	32 (76.2)	
3	11 (20.0)	8 (19.0)	
EgR, n (%)	39 (69.6)	32 (76.2)	0.47
PgR, n (%)	28 (69.6)	29 (69.0)	0.95
HER2 overexpression, n (%)	22 (39.3)	23 (54.8)	0.13
Ki67 > 20%, n (%)	25 (53.2)	23 (57.5)	0.69
Biological subtypes of BC, n (%)			
HR+/HER2-	25 (44.6)	17 (40.5)	
HR-/HER2-	9 (16.1)	3 (7.1)	0.39
HR-/HER2+	8 (14.3)	6 (14.3)	
HR+/HER2+	14 (25.0)	16 (38.1)	
Hypertension, n (%)	31 (55.4)	20 (47.6)	0.45
Diabetes mellitus, n (%)	6 (10.7)	2 (4.8)	0.29
Antithrombotic prophylaxis, n (%)	9 (16.1)	4 (9.5)	0.38

N – number, mean ± standard deviation, median (lower quartile – upper quartile), p – probability value, EgR – estrogen receptor, PgR – progesteron receptor, HER2 – human epidermal growth factor 2, HR – hormone receptors, BC – breast cancer

In the ADJ subgroup 24 (42.9%) patients had factors predicting metastasizing (angioinvasion – N = 5, positive margins – N = 12, adipose tissue invasion – N = 14, invasion of the lymph node capsules – N = 8).
Preoperative therapy was based on doxorubicin or epirubicin with cyclophosphamide with or without taxane (N = 41), 5-fluorouracil with doxorubicin or epirubicin and cyclophosphamide (N = 4), taxane in monotherapy (N = 1), taxane with trastuzumab (N = 2), trastuzumab (N = 5).
Pathological response to preoperative CTH was analyzed in 35 of 42 patients. Complete and partial responses were achieved in 10 (28.6%) and 22 (62.9%) patients, respectively. Stable disease was noted in three women.
A median follow-up period lasted 52 months (quartiles: 37–65 months) in patients on adjuvant therapy and 32 months (quartiles: 26–47 months) on preoperative therapy. During the observation period 6 (12.2%) patients in ADJ subgroup and 5 (12.2%) in NEO-ADJ subgroup had disease progression, while 6 (12.2%) and 2 (5.0%) died, respectively.

Serum levels of VEGF-A, sVEGFR-2, and galectin-3

There were no significant differences in serum levels of VEGF-A, sVEGFR-2, and galectin-3 between patients starting adjuvant and perioperative therapy in univariate analysis (Table II). No association between clinical stage and serum levels of VEGF-A, sVEGFR--2, and galectin-3 was found in the combined study group (Figure 1), as well within subgroup starting perioperative therapy 9.86 (8.65–16.20) vs 14.15 (8.99––18.09) for VEGF-A (p = 0.28), 30.74 (23.40–34.99) vs 29.03 (22.26–34.32) for sVEGFR-2 (p = 0.80), and 35.19 (29.44–58.61) vs 48.50 (34.17–60.76) for galectin-3 (p = 0.16); CS-II vs CS-III respectively.
During therapy, a significant percentage increase of the baseline value in VEGF-A concentration was noted (Table II, Figure 2). It was greater in the subgroup receiving perioperative CTH than adjuvant therapy: 97.7 (10.5–209.4)% vs 58.9 (11.0–104.8)%, however, the difference was not statistically significant in univariate analysis (p = 0.33).

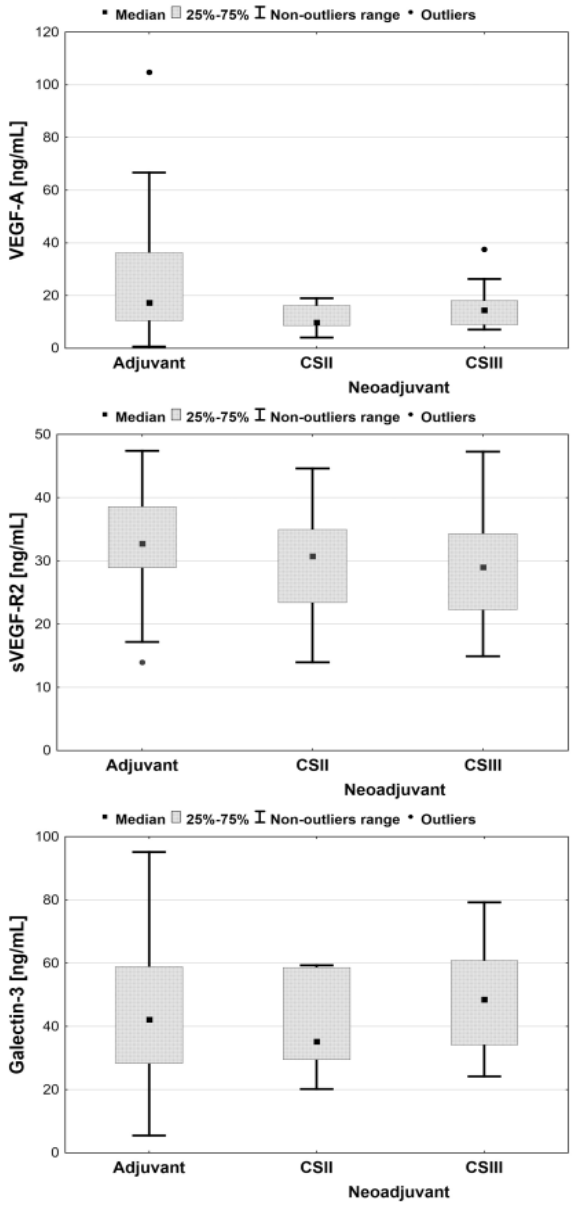

Fig. 1. Serum levels of vascular endothelial growth factor A (VEGF-A), soluble receptor for endothelial growth factor type 2 (sVEGFR-2), and galectin-3 in breast cancer patients starting adjuvant therapy in clinical stages I–III (after tumor and metastatic lymph nodes resection) and patients starting neoadjuvant/induction chemotherapy in clinical stage II and III (two patient-subgroup in CS-I was omitted). No significant changes between subgroups were noted.
Ryc. 1. Stężenie śródnabłonkowego czynnika wzrostu A (VEGF-A), drugiego rozpuszczalnego receptora dla śródnabłonkowego czynnika wzrostu (sVEGFR-2) i galektyny-3 w surowicy pacjentek z rakiem piersi w stadium I–III zaawansowania klinicznego (po usunięciu guza i przerzutów w węzłach chłonnych) rozpoczynających chemioterapię uzupełniającą oraz pacjentek w stadium II i III zaawansowania klinicznego rozpoczynających terapię neoadjuwantową. Nie stwierdzono statystycznie istotnych różnic między grupami.

Table II. Serum concentrations of vascular endothelial growth factor A (VEGF-A), its soluble receptor 2 (sVEGFR-2), and galectin-3 at baseline and after 2 months of follow-up and percentage relative change of the baseline value in patients treated with adjuvant therapy (ADJ) and preoperative chemotherapy (NEO-ADJ)
Tabela II. Stężenie śródnabłonkowego czynnika wzrostu A (VEGF-A), jego rozpuszczalnego receptora 2 (sVEGFR-2) i galektyny-3 w surowicy pacjentek po 2-miesięcznej obserwacji oraz procentowa, względna zmiana ich stężenia w stosunku do wartości wyjściowej u pacjentek leczonych adjuwantową (ADJ) i neoadjuwantową (NEO-ADJ) chemioterapią

Parameters	ADJ	NEO-ADJ	Statistical significance
	N = 56	N = 42	
Baseline values			
VEGF-A [ng/mL]	19.3 (10.5–37.8)	11.8 (8.7–17.4)	0.23
sVEGFR-2 [ng/mL]	32.8 (28.9–38.6)	30.5 (22.7–34.7)	0.15
Galectin-3 [ng/mL]	42.2 (28.2–58.9)	39.3 (31.5–58.9)	0.94
Follow-up values			
VEGF-A [ng/mL]	33.1 (15.5–75.0)	20.3 (13.3–36.1)	0.11
sVEGFR-2 [ng/mL]	35.2 (29.5–40.1)	33.5 (24.0–41.5)	0.18
Galectin-3 [ng/mL]	50.8 (43.1–69.4)	58.4 (42.0–60.5)	0.79
Relative % change			
VEGF-A [%]	58.9 (11.0–104.8)	97.7 (10.5–209.4)	0.33
sVEGFR-2 [%]	7.3 (-8.8–17.5)	13.9 (5.6–22.1)	0.14
Galectin-3 [%]	18.8 (-5.3–75.7)	24.0 (-0.5–47.7)	0.94

median (lower quartile – upper quartile)

One-way analysis of variance with repeated measurements and contrast analysis confirmed no influence of the analyzed subgroup on VEGF-A values ($p = 0.11$), but the statistically significant influence of the time factor ($p < 0.01$). There was no statistically significant interaction between the time factor and subgroups ($p = 0.75$). In both analyzed subgroups values of VEGF-A were significantly higher after follow-up ($p < 0.05$).
Similarly, during therapy, a much smaller increase in sVEGFR-2 was observed. The increase was greater in the NEO-ADJ subgroup (Table II, Figure 2). In ANOVA analysis both the time factor as well as subgroups were statistically significant ($p < 0.05$), yet also here no significant interaction was noted ($p = 0.37$). sVEGFR-2 values increased significantly through time in the NEO-ADJ subgroup ($p < 0.05$) but not in the ADJ one ($p = 0.24$).

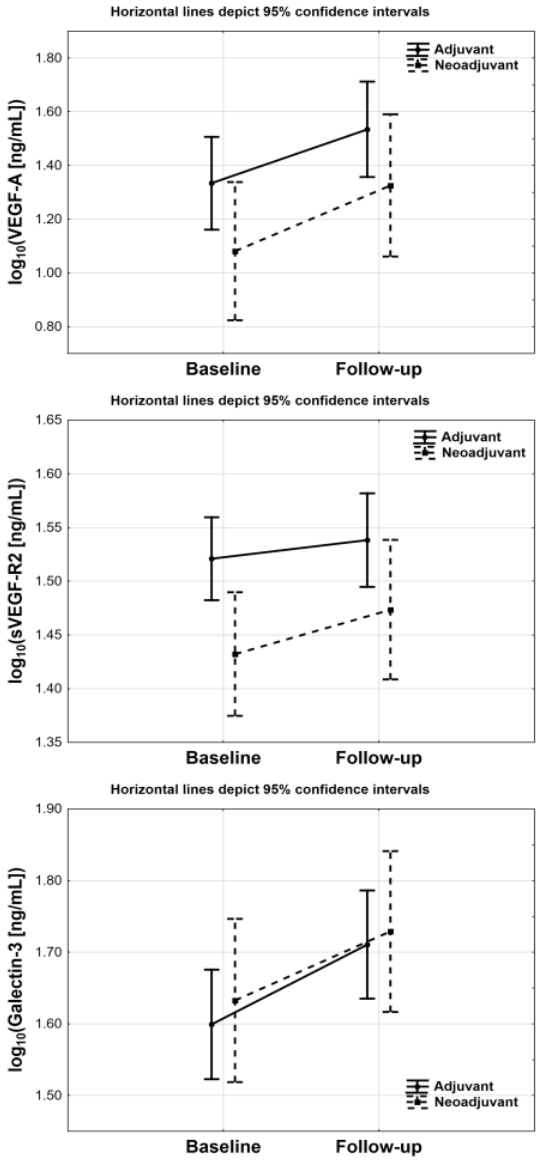

Fig. 2. Changes in serum levels of vascular endothelial growth factor A (VEGF-A), soluble receptor for endothelial growth factor type 2 (sVEGFR--2), and galectin-3 in breast cancer patients during adjuvant therapy and neoadjuvant induction chemotherapy. Significant changes were noted in adjuvant subgroup for VEGF-A and galectin-3, and in neoadjuvant subgroup for VEGF-A, and sVEGFR-2.

Ryc. 2. Zmiany w stężeniu śródnabłonkowego czynnika wzrostu A (VEGF-A), drugiego rozpuszczalnego receptora dla śródnabłonkowego czynnika wzrostu (sVEGFR-2) i galektyny-3 u pacjentek z rakiem piersi leczonych chemioterapią uzupełniającą oraz neoadjuwantową. Istotne statystycznie zmiany uwidoczniono w podgrupie leczonej chemioterapią uzupełniającą dla stężenia VEGF-A i galektyny-3 oraz w podgrupie stosującej terapię neoadjuwantową dla stężenia VEGF-A i sVEGFR-2.

There was also a marked increase in galectin-3 levels during therapy in comparison to baseline value: 18.8 (-5.3–75.7)% in the ADJ subgroup and 24.0 (-0.5––47.7)% in the NEO-ADJ subgroup, yet also here statistically non-significant difference between groups was noted ($p = 0.94$; Figure 2). In ANOVA analysis only the time factor was statistically significant ($p < 0.01$). There were also no differences between groups at baseline ($p = 0.63$) and at follow-up ($p = 0.79$). The increase in galectin-3 values through time was statistically significant in the ADJ subgroup ($p < 0.01$) but not in the NEO-ADJ group ($p = 0.09$).

Prediction of pathological response to preoperative CTH

No predictive significance for obtaining CR was noted for VEGF-A, sVEGFR-2, and galectin-3 in the preoperative group. Similarly changes in VEGF-A, sVEGFR-2, and galectin-3 failed to predict the response (data not shown).

DISCUSSION

In the present study, we evaluated the serum concentration of VEGF, sVEGFR-2, and galectin-3 in patients with stage I-III non-metastatic BC. We did not find any correlation between the serum concentrations of VEGF, sVEGFR-2, and galectin-3 and tumor size, as similar levels were observed in patients starting adjuvant therapy after surgery and at the beginning of preoperative CTH in clinical stages II and III BC. In addition, we found an increase in the VEGF-A, sVEGFR-2 and galectin-3 levels during therapy, both in the NEO-ADJ and ADJ subgroup, but did not observe any relationship between the initial concentration of these cytokines or their changes and pathological response to preoperative CTH.

There are a number of recently published studies attempting to verify the usefulness of the serum concentration of VEGF as a potential BC marker, but to date the results are inconclusive [28]. Several researchers suggested an increased serum concentration of VEGF in patients with stage I-III BC comparing to controls, but the difference was statistically insignificant [16,29]. Similar to our results, a previous study by Stathopoulos et al. [30] did not reveal a correlation between the concentration of VEGF and the size of the tumor, the presence of lymph node metastases, distant metastases, or tumor stage.

In this study, we further investigated the usefulness of VEGF as a predictive marker of BC response to CTH. We demonstrated a significant increase in the VEGF-A concentration during therapy, greater in the subgroup receiving preoperative CTH than adjuvant therapy. The results, however, did not indicate an association between the VEGF concentration and tumor response in the group of patients undergoing neoadjuvant/induction CTH. Our observations are not in line with other studies. Wang et al. [31] discovered in a large group of triple-negative BC ($N = 303$) a predictive value of the relative change in serum VEGF before the third cycle of neoadjuvant CTH. The decrease in serum VEGF had a predictive value for pathological complete

response and correlated with DFS. These findings suggested the usefulness of VEGF serum monitoring in identifying patients responding to neoadjuvant CTH in patients with triple-negative BC. Of note, we did not observe a decrease in serum VEGF in our small group of patients with mostly luminal BC obtaining pCR.

In one of the previously published studies, the authors attempted to evaluate serum concentrations of angiogenic factors in patients with locally advanced BC during neoadjuvant CTH, including VEGF. They revealed a statistically insignificant increased serum VEGF level in BC patients, with a transient increase in this cytokine concentration during the treatment (in the first two cycles), and a return to the basal level before surgical treatment [32]. Winter et al. [33] also evaluated the serum VEGF concentration in 2 subgroups of non--metastatic BC patients treated by means of neoadjuvant CTH with or without zoledronic acid (total N = 39). They found no difference in the VEGF level on the 21st day of treatment and before surgery, with a transient decrease in this cytokine concentration on the 5th day of treatment in the zoledronic acid subgroup. Nonetheless the impact of zoledronic acid on the VEGF concentration, and consequently its antiangiogenic effect, require more investigations. The presented differences may result from the different time-points of sampling and methodology of VEGF measurements.

Few studies assessed the clinical usefulness of sVEGFR-2 in women with BC. We found a noticeable increase in the sVEGFR-2 concentration during both neoadjuvant and adjuvant CTH but failed to demonstrate an association with the effectiveness of treatment and the clinical stage. Nevertheless, most of the previously published studies presented correlations between the tumor stage or the presence of metastases and the serum concentration of sVEGFR-2 in patients with BC [16,28,34]. The discrepancies may result from a relatively weak association, the influence of age/menopausal status [34], varying histological grading [34], and the small sizes of the study cohorts (the largest included 103 patients). It was acknowledged that sVEGF may serve as natural antagonists of neoangiogenesis in BC. Thus, increased concentrations of sVEGFR-2 could be considered a natural defense mechanism against tumoral angiogenesis [28]. Therefore, it was postulated that an increased serum concentration of sVEGFR-2 could have a positive prognostic significance and may predict a longer cancer-specific survival [34]. However, our study does not support this hypothesis.

To date, there is little evidence on the role of galectin-3 as a potential marker and prognostic factor in patients with BC. Iurisci et al. [21] were the first to suggest a higher serum concentration of galectin-3 in patients with BC. Of note, the results were not statistically significant. This observation was supported by Topcu et al. [22] who demonstrated significantly higher serum levels of galectin-3 in patients with BC than in controls. The authors attempt to establish the cut-off point of galectin-3 to predict BC occurrence at ≥ 3.17 ng/ml. However, the sensitivity and specificity of this estimation were moderate. Contrary to the above--mentioned results, the latest findings did not confirm increased circulating galectin-3 levels in patients with BC compared to healthy populations [23].

In our study, the serum concentration of galectin-3 was increased during both neoadjuvant/induction and adjuvant CTH. However, the increase in the galectin-3 concentration did not correlate with the effectiveness of CTH. Galectin-3 expression was shown to play a protective role in BC cell survival [35,36]. On the other hand, it is difficult to expect that circulating concentrations would reflect the local expression of galectin-3 in the cancer tissue and the effectiveness of the therapy in destroying neoplastic cells. Some authors put forward a thesis that CTH, by increasing the concentration of galectin-3, would cause more intense apoptosis of neoplastic cells [23]. Only a few studies found a correlation between the increased serum concentration of galectin-3 and the effectiveness of applied therapy [23,37], which made the prognostic role of galectin-3 for the effectiveness of CTH in patients with BC unlikely. Our study is one of those with negative findings.

Study limitations

This study has several limitations, related to the size of our single-center cohort, precluding analysis of biological subtypes. Consistent with cancer statistics, most of our patients had luminal cancer subtypes, and therefore the results of the analysis was dominated by this subtype. Our study should be considered preliminary. However, the negative character of the results, which is in line with other published studies, reduces the enthusiasm to perform larger, multicenter clinical trials.

CONCLUSIONS

1. The serum levels of VEGF-A, sVEGFR-2, and galectin-3 do not correlate with the clinical stage or tumor size in patients with non-metastatic breast cancer.

2. The baseline levels of VEGF-A, sVEGFR-2, and galectin-3 and the observed increase in the levels of VEGF-A and sVEGFR-2 during preoperative chemotherapy do not predict its efficacy.

Funding

This study was funded by the Medical University of Silesia, Katowice, Poland. Project No. KNW-2-K59/D/6/K, KNW-2-055/D/5/N.

Author's contribution

Study design – J. Wojnar, T. Francuz, I. Grochoła-Małecka
Data collection – I. Grochoła-Małecka, G. Handzlik
Methodology – T. Francuz, P. Czajka-Francuz, I. Grochoła-Małecka
Statistical analysis – A.J. Owczarek
Manuscript preparation – J. Chudek, I. Grochoła-Małecka
Literature research – I. Grochoła-Małecka, G. Handzlik, J. Wojnar

Supervision – J. Chudek

REFERENCES

1. Estimated age-standardized incidence and mortality rates (World) in 2020, World, both sexes, all ages (excl. NMSC) (bar chart). Global Cancer Observatory, 2020 [online] <https://gco.iarc.fr/today/online-analysis-multi-bars?v=2020&mode=cancer&mode_population=countries&population=900&populations=900&key=asr&sex=0&cancer=39&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=10&group_cancer=1&include_nmsc=0&include_nmsc_other=1&type_multiple=%257B%2522inc%2522%253Atrue%252C%2522mort%2522%253Atrue%252C%2522prev%2522%253Afalse%257D&orientation=horizontal&type_sort=0&type_nb_items=%257B%2522top%2522%253Atrue%252C%2522bottom%2522%253Afalse%257D> [accessed on 27 September 2022].
2. Weidner N., Semple J.P., Welch W.R., Folkman J. Tumor angiogenesis and metastasis–correlation in invasive breast carcinoma. N. Engl. J. Med. 1991; 324(1): 1–8, doi: 10.1056/NEJM199101033240101.
3. Rykala J., Przybylowska K., Majsterek I., Pasz-Walczak G., Sygut A., Dziki A. et al. Angiogenesis markers quantification in breast cancer and their correlation with clinicopathological prognostic variables. Pathol. Oncol. Res. 2011; 17(4): 809–817, doi: 10.1007/s12253-011-9387-6.
4. Gao S., Ma J.J., Lu C. Prognostic significance of VEGF-C immunohistochemical expression in breast cancer: a meta-analysis. Tumour Biol. 2014; 35(2): 1523–1529, doi: 10.1007/s13277-013-1211-3.
5. Regenfuss D., Cursiefen C. Concept of angiogenic privilege. In: D.A. Dartt, J.C. Besharse, R. Dana (eds.). Encyclopedia of the eye. Vol. 1. Academic Press. Oxford 2010, pp. 334–338.
6. Massena S., Christoffersson G., Vågesjö E., Seignez C., Gustafsson K., Binet F. et al. Identification and characterization of VEGF-A-responsive neutrophils expressing CD49d, VEGFR1, and CXCR4 in mice and humans. Blood 2015; 126(17): 2016–2026, doi: 10.1182/blood-2015-03-631572.
7. Seetharam L., Gotoh N., Maru Y., Neufeld G., Yamaguchi S., Shibuya M. A unique signal transduction from FLT tyrosine kinase, a receptor for vascular endothelial growth factor VEGF. Oncogene 1995; 10(1): 135–147.
8. Ning Q., Liu C., Hou L., Meng M., Zhang X., Luo M. et al. Vascular endothelial growth factor receptor-1 activation promotes migration and invasion of breast cancer cells through epithelial-mesenchymal transition. PLoS One 2013; 8(6): e65217, doi: 10.1371/journal.pone.0065217.
9. Dhakal H.P, Naume B., Synnestvedt M., Borgen E., Kaaresen R., Schlichting E. et al. Expression of vascular endothelial growth factor and vascular endothelial growth factor receptors 1 and 2 in invasive breast carcinoma: prognostic significance and relationship with markers for aggressiveness. Histopathology 2012; 61(3): 350–364, doi: 10.1111/j.1365-2559.2012.04223.x.
10. Arias-Pulido H., Chaher N., Gong Y., Qualls C., Vargas J., Royce M. Tumor stromal vascular endothelial growth factor A is predictive of poor outcome in inflammatory breast cancer. BMC Cancer 2012; 12: 298, doi: 10.1186/1471-2407-12-298.
11. Failla C., Carbo M., Morea V. Positive and negative regulation of angiogenesis by soluble vascular endothelial growth factor receptor-1. Int. J. Mol. Sci. 2018; 19(5): 1306, doi: 10.3390/ijms19051306.
12. Stevens M., Oltean S. Modulation of receptor tyrosine kinase activity through alternative splicing of ligands and receptors in the VEGF-A/VEGFR axis. Cells 2019; 8(4): 288, doi: 10.3390/cells8040288.
13. Autenshlyus A., Arkhipov S., Mikhailova E., Arkhipova V., Varaksin N. VEGF-R2 and TNF-R1 expression and cytokine production by samples of mammary adenocarcinomas and correlations with histopathological parameters of these malignant tumors. Int. J. Immunopathol. Pharmacol. 2018; 32: 2058738418787990, doi: 10.1177/2058738418787990.
14. Zajkowska M., Lubowicka E., Fiedorowicz W., Szmitkowski M., Jamiołkowski J., Ławicki S. Human plasma levels of VEGF-A, VEGF-C, VEGF-D, their soluble receptor – VEGFR-2 and applicability of these parameters as tumor markers in the diagnostics of breast cancer. Pathol. Oncol. Res. 2019; 25(4): 1477–1486, doi: 10.1007/s12253-018-0527-0.
15. Toi M., Bando H., Ogawa T., Muta M., Hornig C., Weich H.A. Significance of vascular endothelial growth factor (VEGF)/soluble VEGF receptor-1 relationship in breast cancer. Int. J. Cancer 2002; 98(1): 14–18, doi: 10.1002/ijc.10121.
16. Thielemann A., Baszczuk A., Kopczyński Z., Kopczyński P., Grodecka-Gazdecka S. Clinical usefulness of assessing VEGF and soluble receptors sVEGFR-1 and sVEGFR-2 in women with breast cancer. Ann. Agric. Environ. Med. 2013; 20(2): 293–297.
17. Yang R.Y., Rabinovich G.A., Liu F.T. Galectins: structure, function and therapeutic potential. Expert Rev. Mol. Med. 2008; 10: e17, doi: 10.1017/S1462399408000719.
18. Menon R.P., Hughes R.C. Determinants in the N-terminal domains of galectin-3 for secretion by a novel pathway circumventing the endoplasmic reticulum-Golgi complex. Eur. J. Biochem. 1999; 264(2): 569–576, doi: 10.1046/j.1432-1327.1999.00671.x.
19. Yu L.G. Circulating galectin-3 in the bloodstream: An emerging promoter of cancer metastasis. World J. Gastrointest. Oncol. 2010; 2(4): 177–180, doi: 10.4251/wjgo.v2.i4.177.
20. Yamaki S., Fujii T., Yajima R., Hirakata T., Yamaguchi S., Fujisawa T. et al. Clinicopathological significance of decreased galectin-3 expression and the long-term prognosis in patients with breast cancer. Surg. Today 2013; 43(8): 901–905, doi: 10.1007/s00595-012-0378-3.
21. Iurisci I., Tinari N., Natoli C., Angelucci D., Cianchetti E., Iacobelli S. Concentrations of galectin-3 in the sera of normal controls and cancer patients. Clin. Cancer Res. 2000; 6(4): 1389–1393.
22. Topcu T.O., Kavgaci H., Gunaldi M., Akyol M., Mentese A., Yaman S.O. et al. The clinical importance of serum galectin-3 levels in breast cancer patients with and without metastasis. J. Cancer Res. Ther. 2018; 14(10): S583––S586, doi: 10.4103/0973-1482.176425.
23. Shafiq A., Moore J., Suleman A., Faiz S., Farooq O., Arshad A. et al. Elevated soluble galectin-3 as a marker of chemotherapy efficacy in breast cancer patients: A prospective study. Int. J. Breast Cancer 2020; 2020: 4824813, doi: 10.1155/2020/4824813.
24. Giuliano A.E., Connolly J.L., Edge S.B., Mittendorf E.A., Rugo H.S., Solin L.J. et al. Breast cancer-major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J. Clin. 2017; 67(4): 290–303, doi: 10.3322/caac.21393.
25. Singh K., Tantravahi U., Lomme M.M., Pasquariello T., Steinhoff M., Sung C.J. Updated 2013 College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guideline recommendations for human epidermal growth factor receptor 2 (HER2) fluorescent in situ hybridization (FISH) testing increase HER2 positive and HER2 equivocal breast cancer cases; retrospective study of HER2 FISH results of 836 invasive breast cancers. Breast Cancer Res. Treat. 2016; 157(3): 405–411, doi: 10.1007/s10549-016-3824-x.
26. AJCC cancer staging manual. 8th ed. Amin B.A., Edge S.B., Greene F.L., Byrd D.R., Brookland R.K., Washington M.K. et al. (eds.). Springer. New York 2017, doi 10.1007/978-3-319-40618-3_48.

27. Pinder S.E., Provenzano E., Earl H., Ellis I.O. Laboratory handling and histology reporting of breast specimens from patients who have received neoadjuvant chemotherapy. Histopathology 2007; 50(4): 409–417, doi: 10.1111/j.1365-2559.2006.02419.x.
28. El Tarhouny S., Seefeld M., Fan A.X., Hahn S., Holzgreve W., Zhong X.Y. Comparison of serum VEGF and its soluble receptor sVEGFR1 with serum cell-free DNA in patients with breast tumor. Cytokine 2008; 44(1): 65––69, doi: 10.1016/j.cyto.2008.06.008.
29. Hodorowicz-Zaniewska D., Kibil W., Małek A., Szpor J., Kulig J., Sztefko K. Evaluation of serum concentrations of vascular endothelial growth factor (VEGF) in breast cancer patients. Pol. J. Pathol. 2012; 63(4): 255–260, doi: 10.5114/pjp.2012.32773.
30. Stathopoulos J., Armakolas A., Stathopoulos G.P., Gomatos I.P. Plasma VEGF levels in breast cancer patients with and without metastases. Oncol. Lett. 2010; 1(4): 739–741, doi: 10.3892/ol_00000129.
31. Wang R.X., Chen S., Huang L., Zhou Y., Shao Z.M. Monitoring serum VEGF in neoadjuvant chemotherapy for patients with triple-negative breast cancer: A new strategy for early prediction of treatment response and patient survival. Oncologist 2019; 24(6): 753–761, doi: 10.1634/theoncologist.2017-0602.
32. Fürstenberger G., von Moos R., Lucas R., Thürlimann B., Senn H.J., Hamacher J. et al. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. Br. J. Cancer 2006; 94(4): 524–531, doi: 10.1038/sj.bjc.6602952.
33. Winter M.C., Wilson C., Syddall S.P., Cross S.S., Evans A., Ingram C.E. et al. Neoadjuvant chemotherapy with or without zoledronic acid in early breast cancer–a randomized biomarker pilot study. Clin. Cancer Res. 2013; 19(10): 2755–2765, doi: 10.1158/1078-0432.CCR-12-3235.
34. Zarychta E., Rhone P., Bielawski K., Michalska M., Rość D., Ruszkowska-Ciastek B. Anti-angiogenic efficacy in invasive breast carcinoma patients depends on clinicopathological determinants. Adv. Med. Sci. 2019; 64(2): 216–223, doi: 10.1016/j.advms.2019.02.001.
35. Boutas I., Potiris A., Brenner W., Lebrecht A., Hasenburg A., Kalantaridou S. et al. The expression of galectin-3 in breast cancer and its association with chemoresistance: a systematic review of the literature. Arch. Gynecol. Obstet. 2019; 300(5): 1113–1120, doi: 10.1007/s00404-019-05292-9.
36. Boutas I., Potiris A., Makrakis E., Messaropoulos P., Papaioannou G., Kalantaridou S. The expression of Galectin-3 in breast cancer and its association with metastatic disease: a systematic review of the literature. Mol. Biol. Rep. 2021; 48(1): 807–815, doi: 10.1007/s11033-020-06122-x.
37. De Luliis F., Salerno G., Taglieri L., Lanza R., Cardelli P., Scarpa S. Circulating neuregulin-1 and galectin-3 can be prognostic markers in breast cancer. Int. J. Biol. Markers 2017; 32(3): e333–e336, doi: 10.5301/ijbm.5000262.